

第26回日本臨床細胞学会関東連合会

脳腫瘍病理診断の要点

中里 洋一

群馬大学大学院医学系研究科病態病理学分野

(2012年 9月 8日, 高崎市)

脳腫瘍

- 定義：頭蓋腔内に発生する腫瘍
- 特徴
 - 発生頻度は低い。
 - 腫瘍型が多い。
 - 良性型～悪性型
 - びまん性に浸潤するものがある。
 - 中枢神経機能を障害：痙攣発作、巣症状
 - 頭蓋内圧亢進、水頭症、脳脊髄液播種
 - 膠芽腫の累積生存率は、1年 55.2%，5年 7.0%



脳腫瘍の診断と分類

- 脳腫瘍の研究, 治療には → 診断が必要
 - 腫瘍型が多い: 133種類
 - 生物学的性質が多様: 良性～悪性腫瘍
- 診断の根拠: 分類体系
 - 形態の類似性, 組織発生, 悪性度
- 分類の標準化
 - WHO分類(2007年)
 - 脳腫瘍取扱い規約分類(2010年)

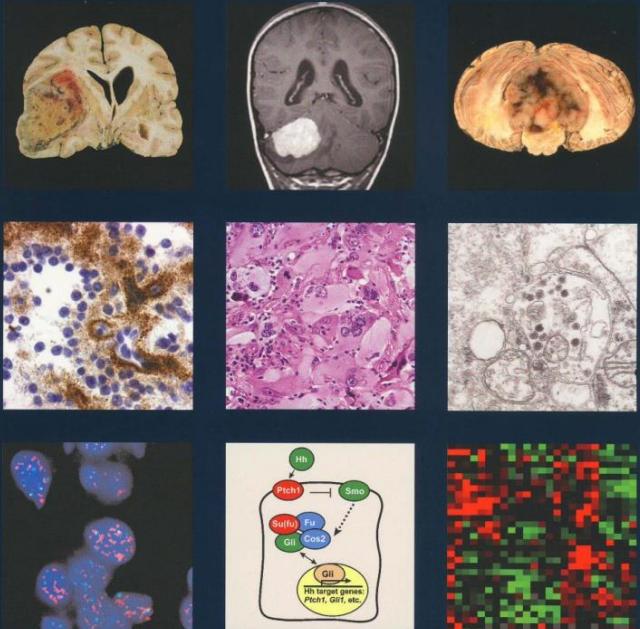
WNO Consensus Meeting



DKFZ in Heidelberg, Nov 17 & 18, 2006

WHO Classification of Tumours of the Central Nervous System

Edited by David N. Louis, Hiroko Ohgaki, Otmar D. Wiestler, Webster K. Cavenee



WHO2007

臨床・病理 脳腫瘍取扱い規約

臨床と病理カラーアトラス

General Rules for Clinical and Pathological Studies on Brain Tumors

2010年7月

第3版

社団日本脳神経外科学会・社団日本病理学会・編

Third Edition, 2010
The Japan Neurosurgical Society
The Japanese Society of Pathology

金原出版株式会社

脳腫瘍取扱い規約

脳腫瘍の診断

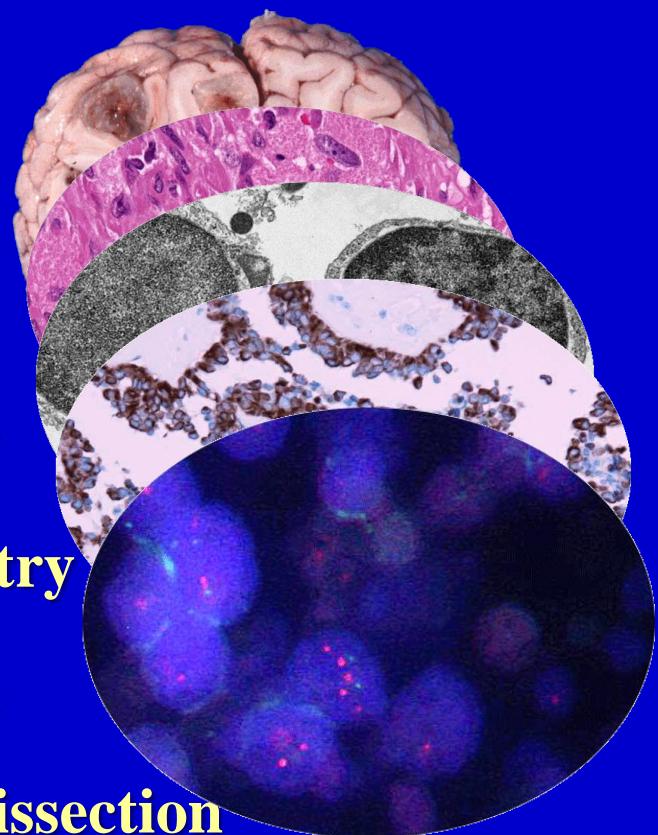
- 診断のtarget
 - 腫瘍か、非腫瘍か？
 - どの細胞に由来する腫瘍か？
 - 良性か、悪性か？
- 診断のmodality
 - 臨床的診断
 - 画像診断
 - 病理学的診断
 - 分子遺伝学的診断

病理学的診断

- 病理形態学：「病気を細胞・組織・器官の形態異常として把握し解析する学問」
- 形態学的診断の特徴
 - 形態の持つ情報量は極めて大きい
 - ヒトの視覚情報処理(パターン認識)を利用
 - 静的情報なので繰り返して解析が可能

脳腫瘍病理診断:技術の進歩

- 19世紀 **Macroscopy**
 Light microscopy
- 20世紀 **Histochemistry**
 Electron microscopy
 Immunohistochemistry
- 21世紀 **In situ hybridization**
 FISH, CGH, microdissection

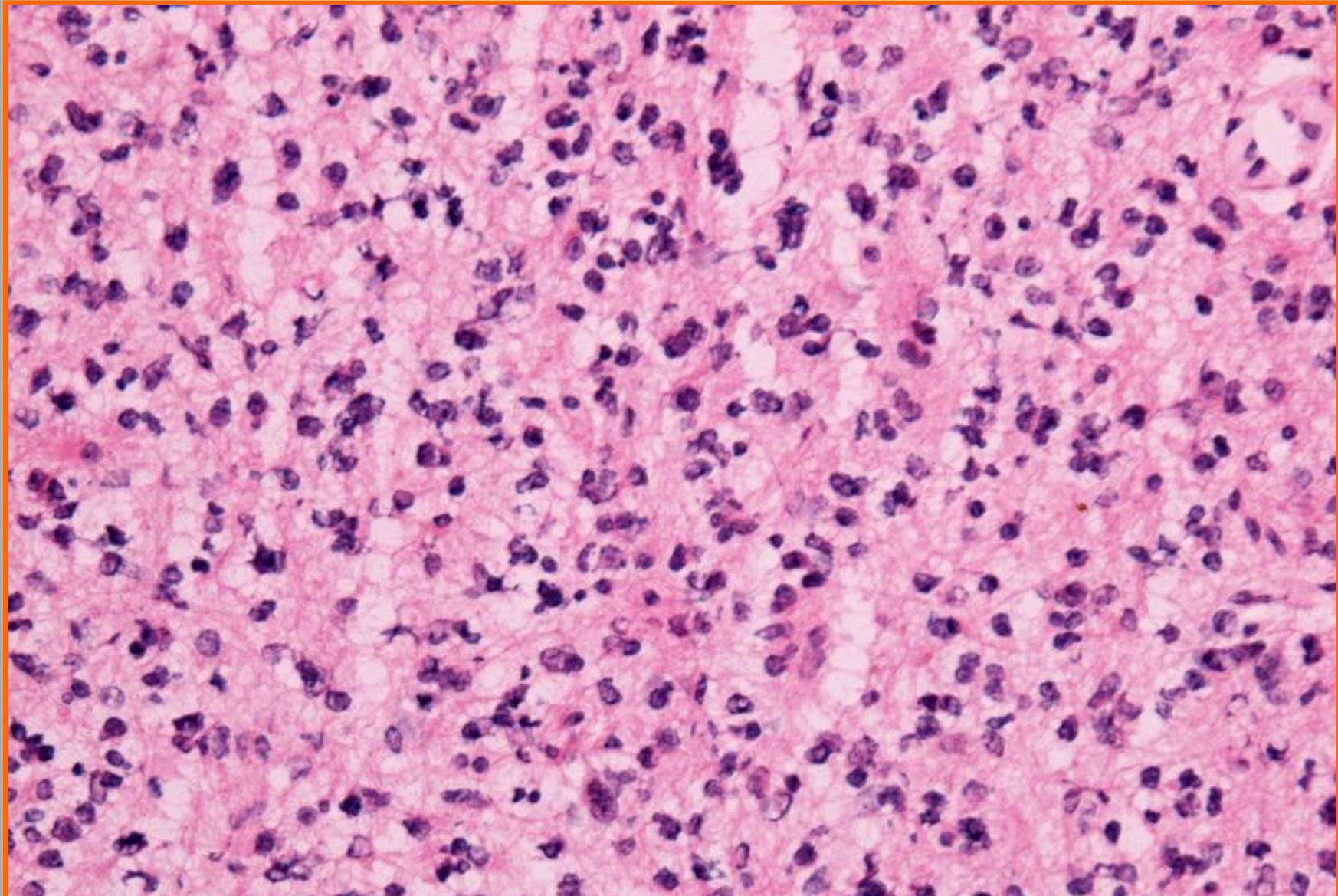


脳腫瘍の病理診断の実際

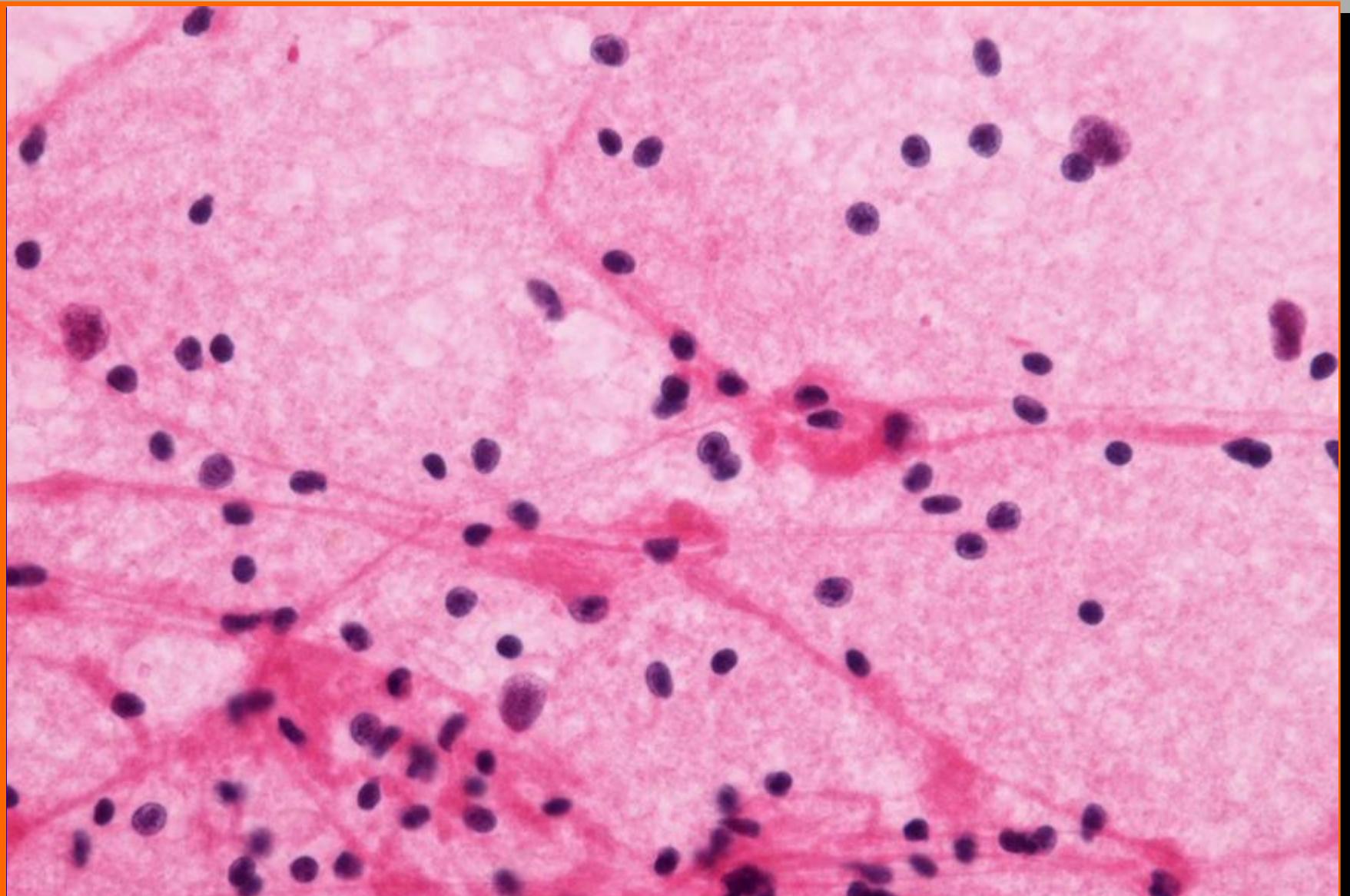
- 目的：腫瘍か？、由来は？、悪性か？
- 検体の種類
 - 術中迅速／手術・生検／剖検
- 検索方法：
 - Light microscopy
 - Immunohistochemistry
 - (Electron microscopy)
 - Genetic analysis
- 診断結果：腫瘍名 + WHO grade

脳腫瘍の術中迅速診断

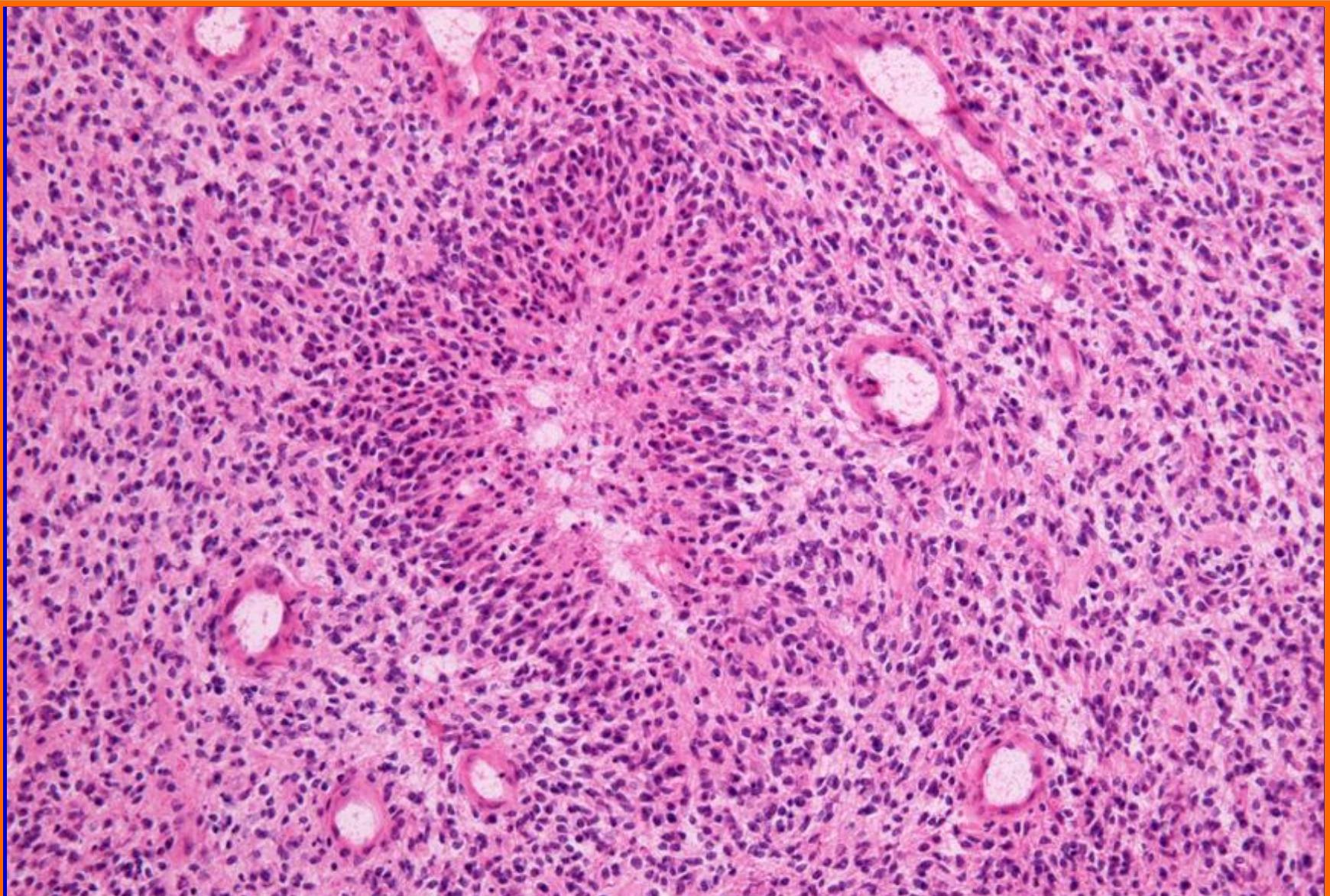
- 迅速性と正確な診断が要求される。
- 組織標本(クリオスタッフ)と
細胞標本(塗沫法、圧挫法)を併用する。
- 診断により手術方針が変更される腫瘍
 - glioblastoma vs malignant lymphoma vs metastatic tumor vs abscess
 - germinoma vs mixed germ cell tumor



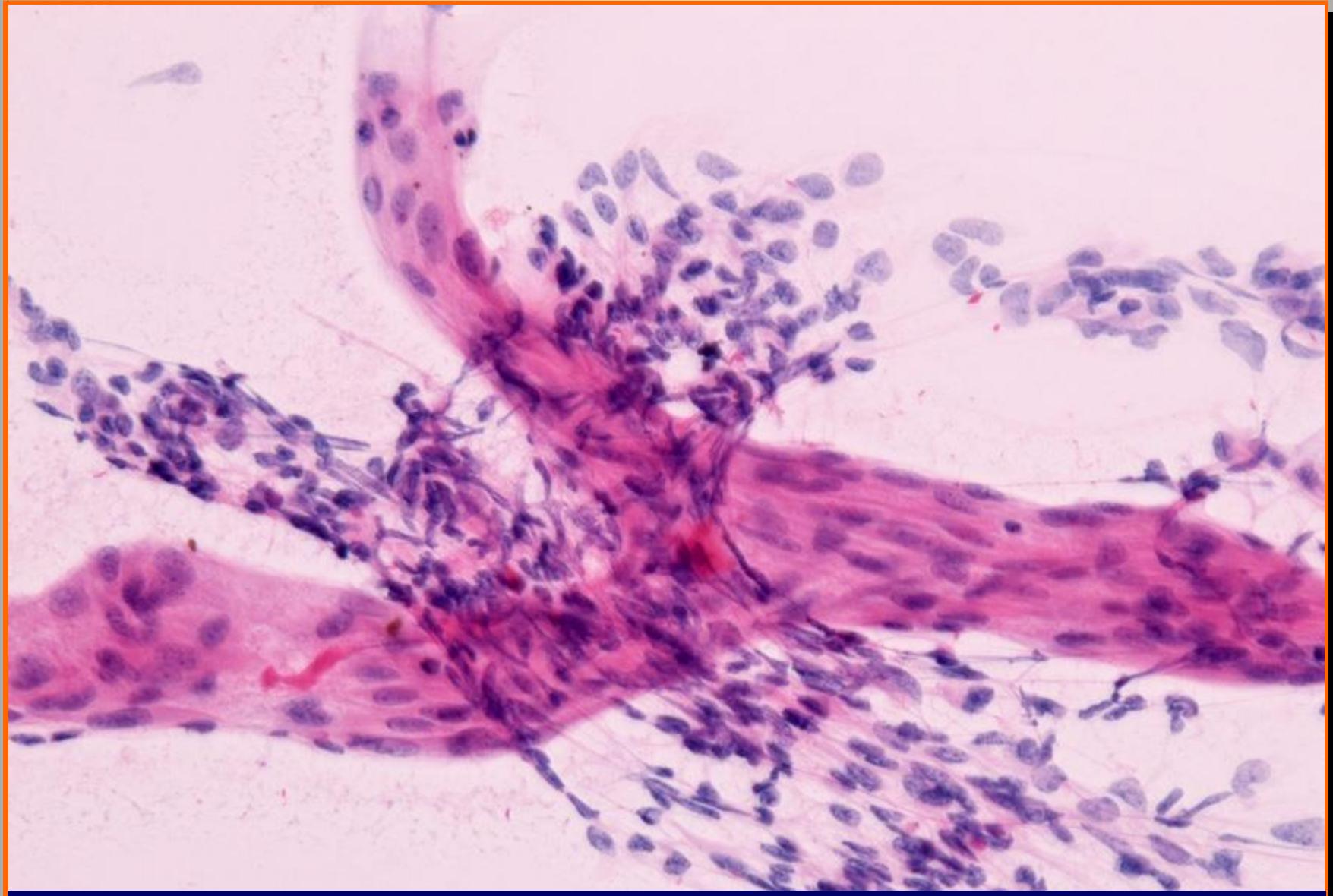
Oligodendrogloma, frozen section



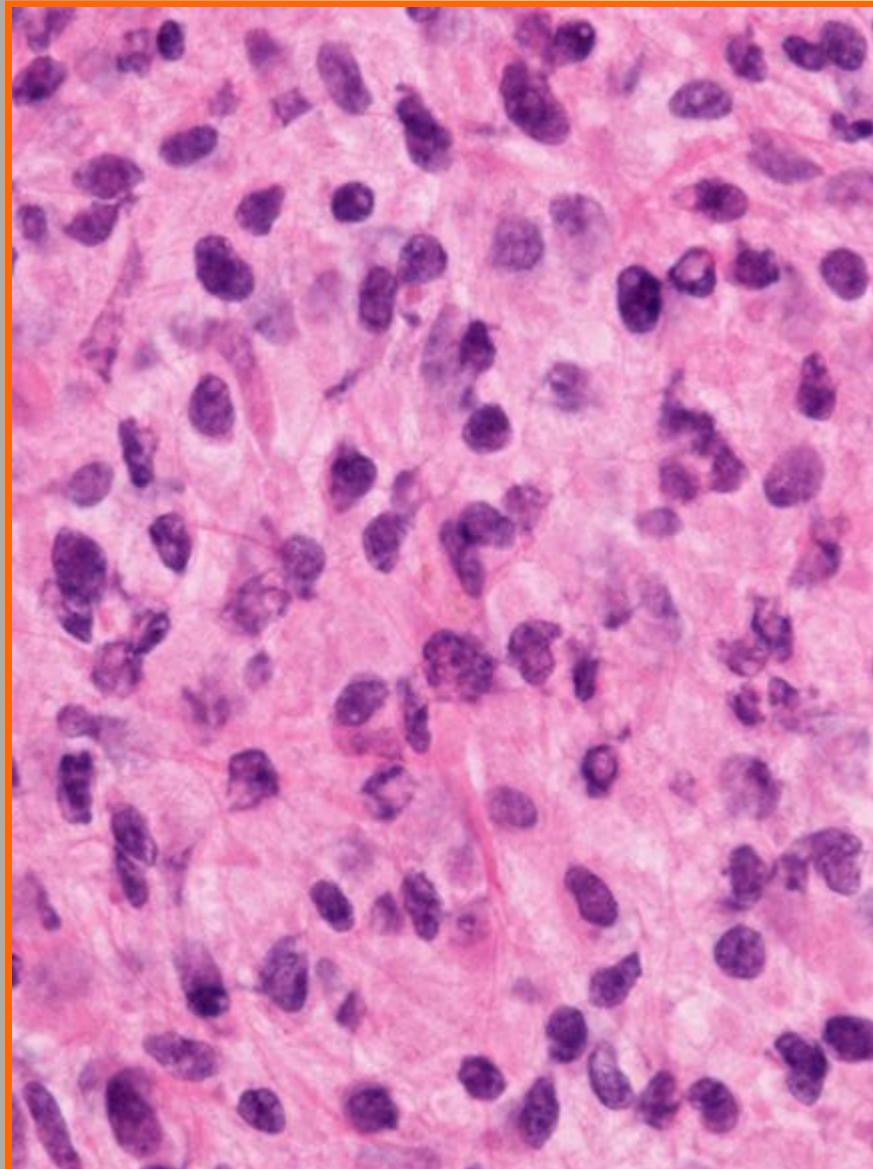
Oligodendrogloma, smear preparation



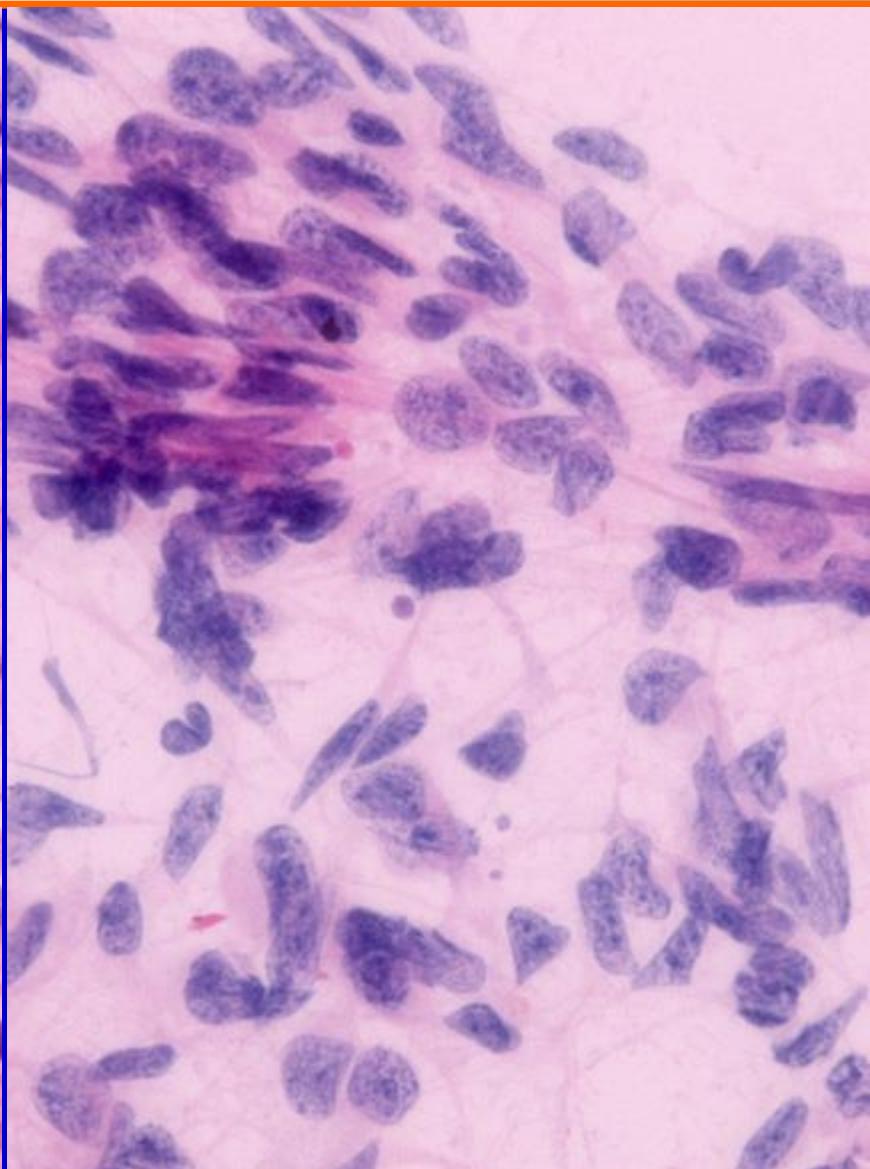
GBM, frozen section



GBM, smear preparation

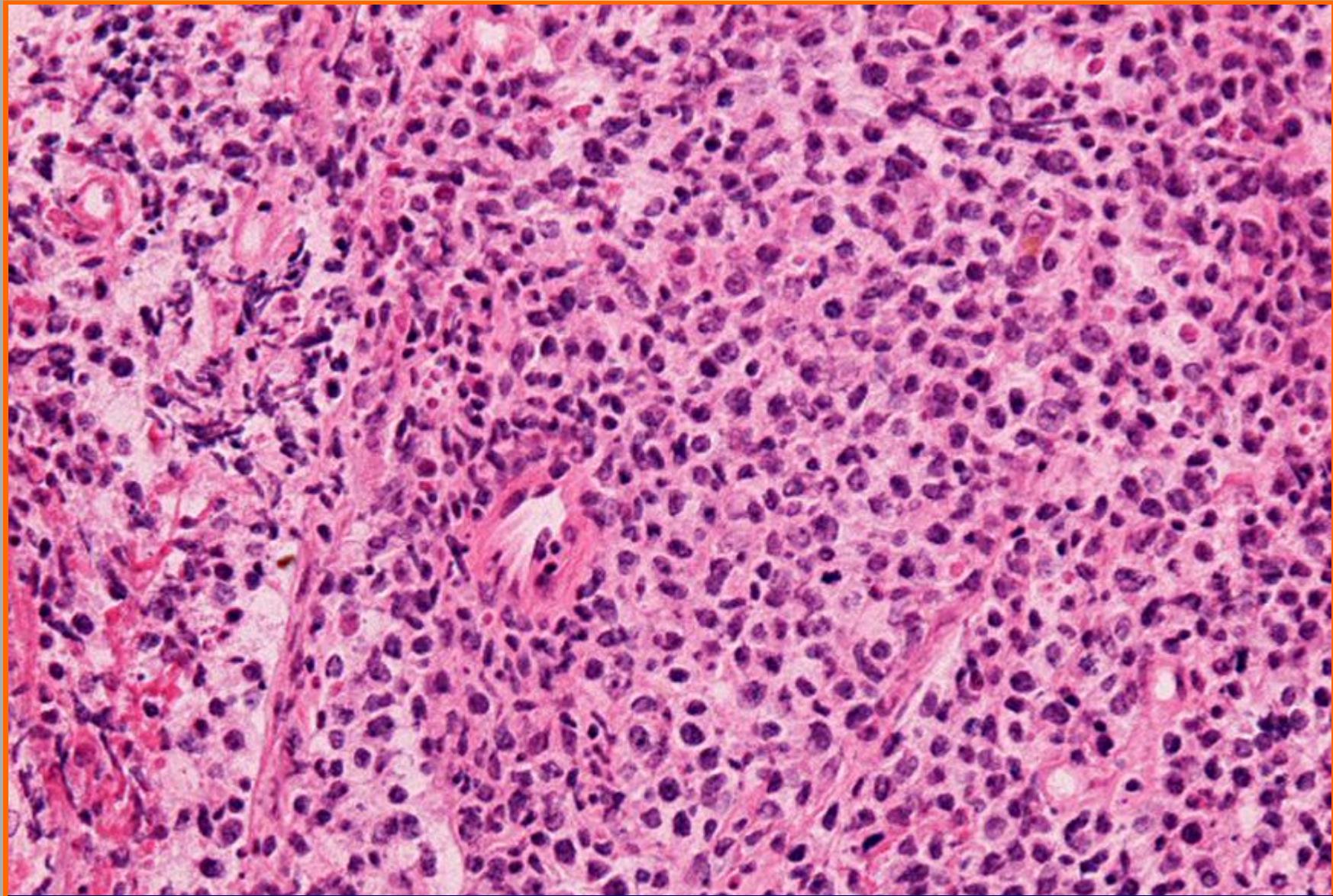


frozen section

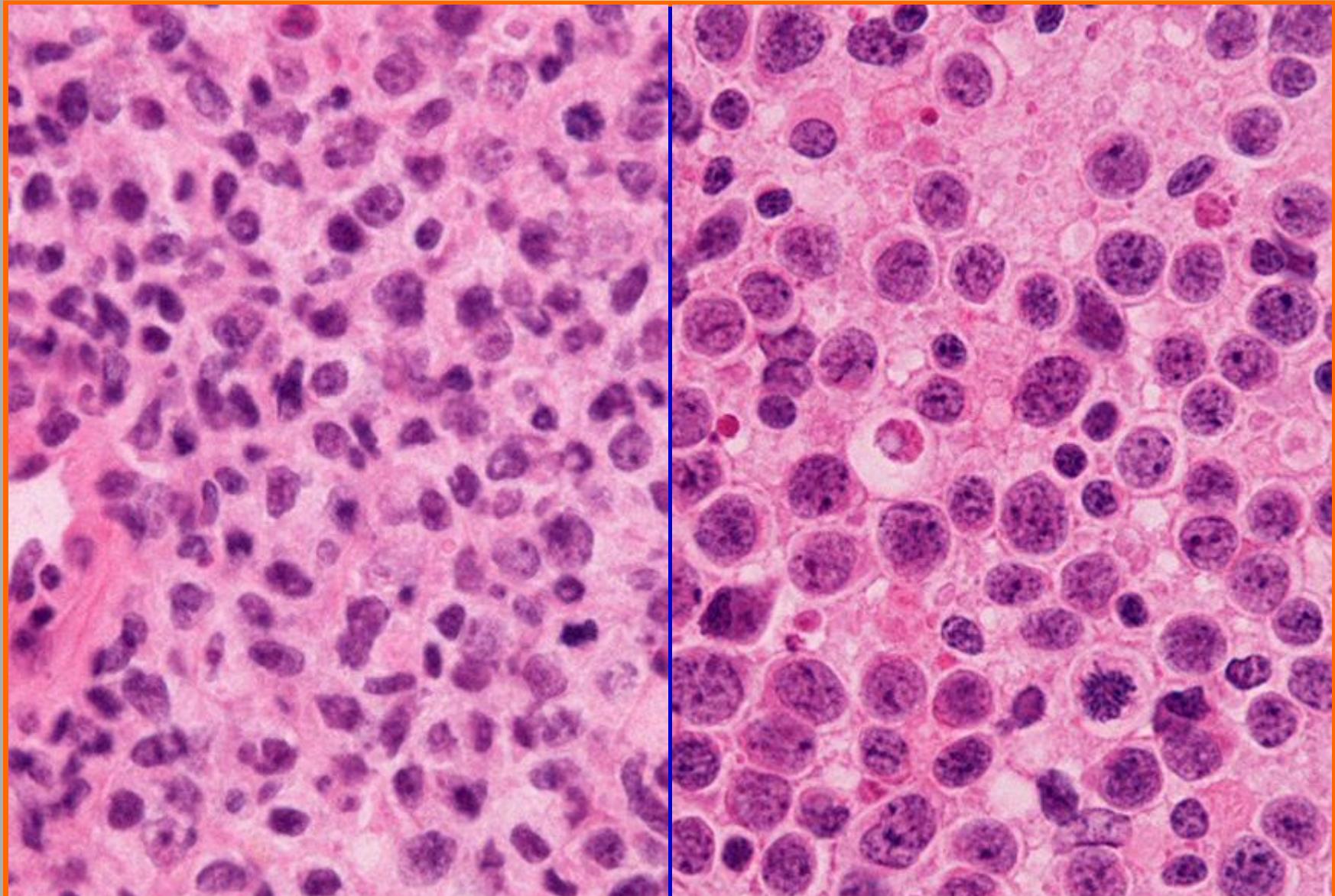


GBM

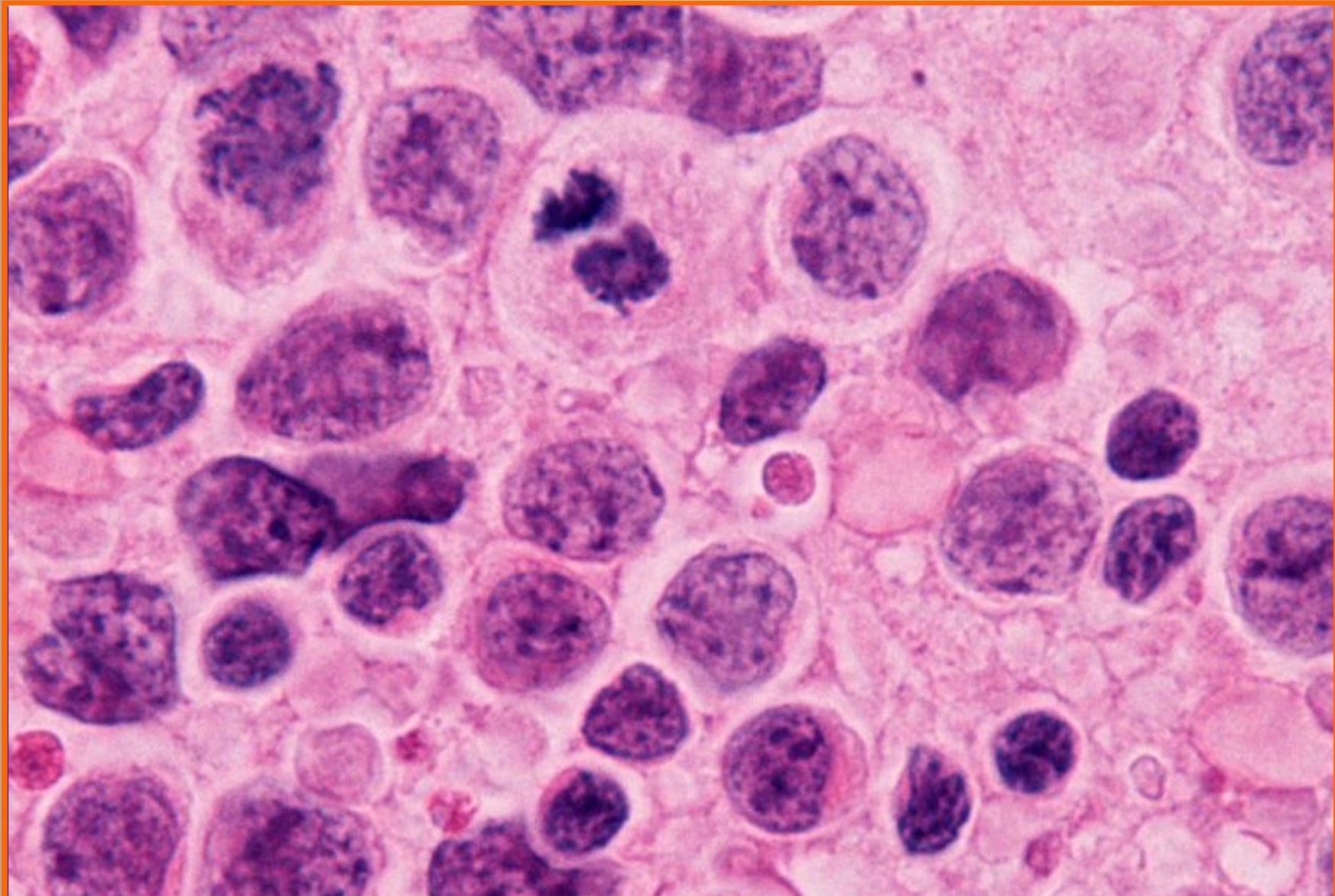
smear preparation



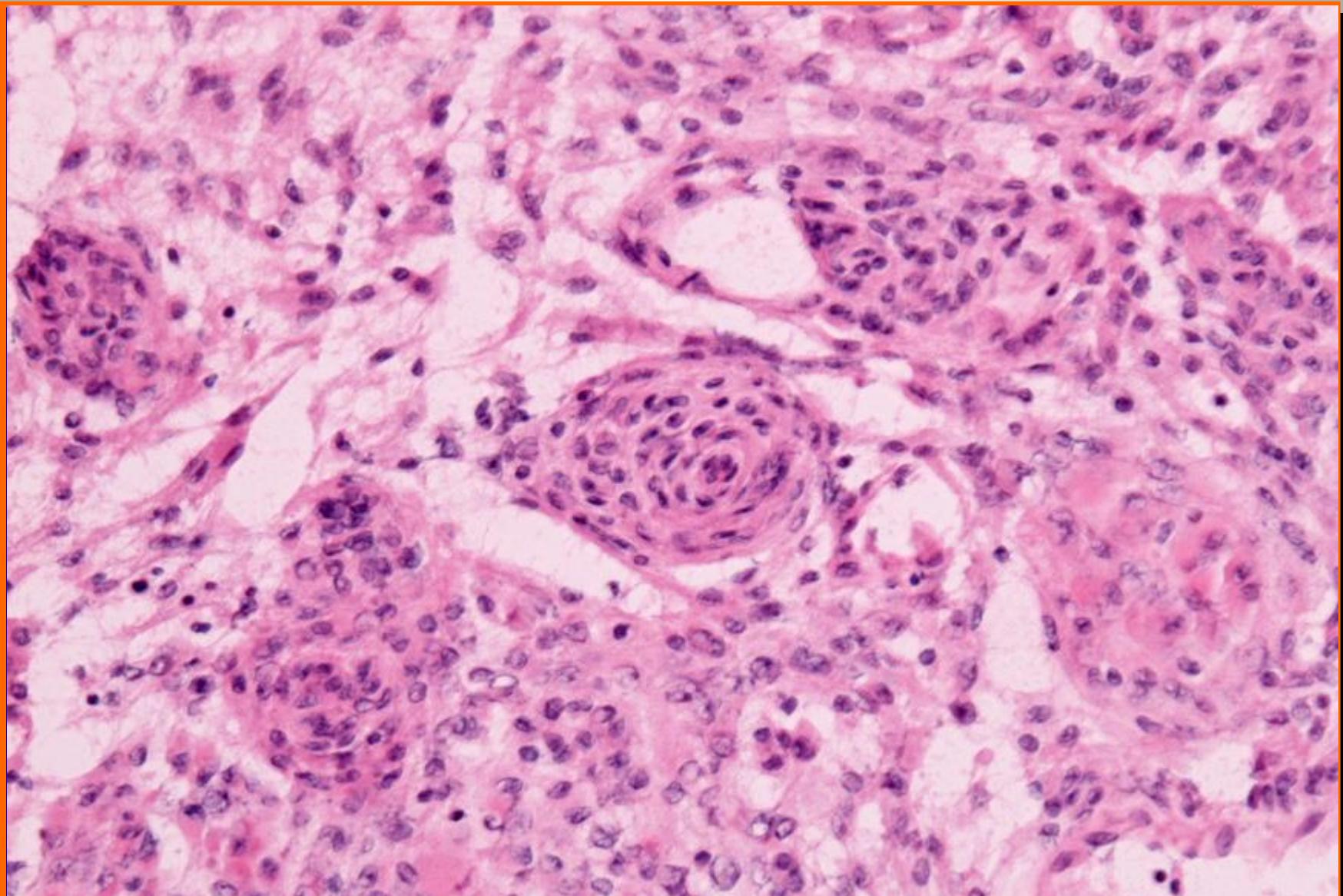
Lymphoma, frozen section



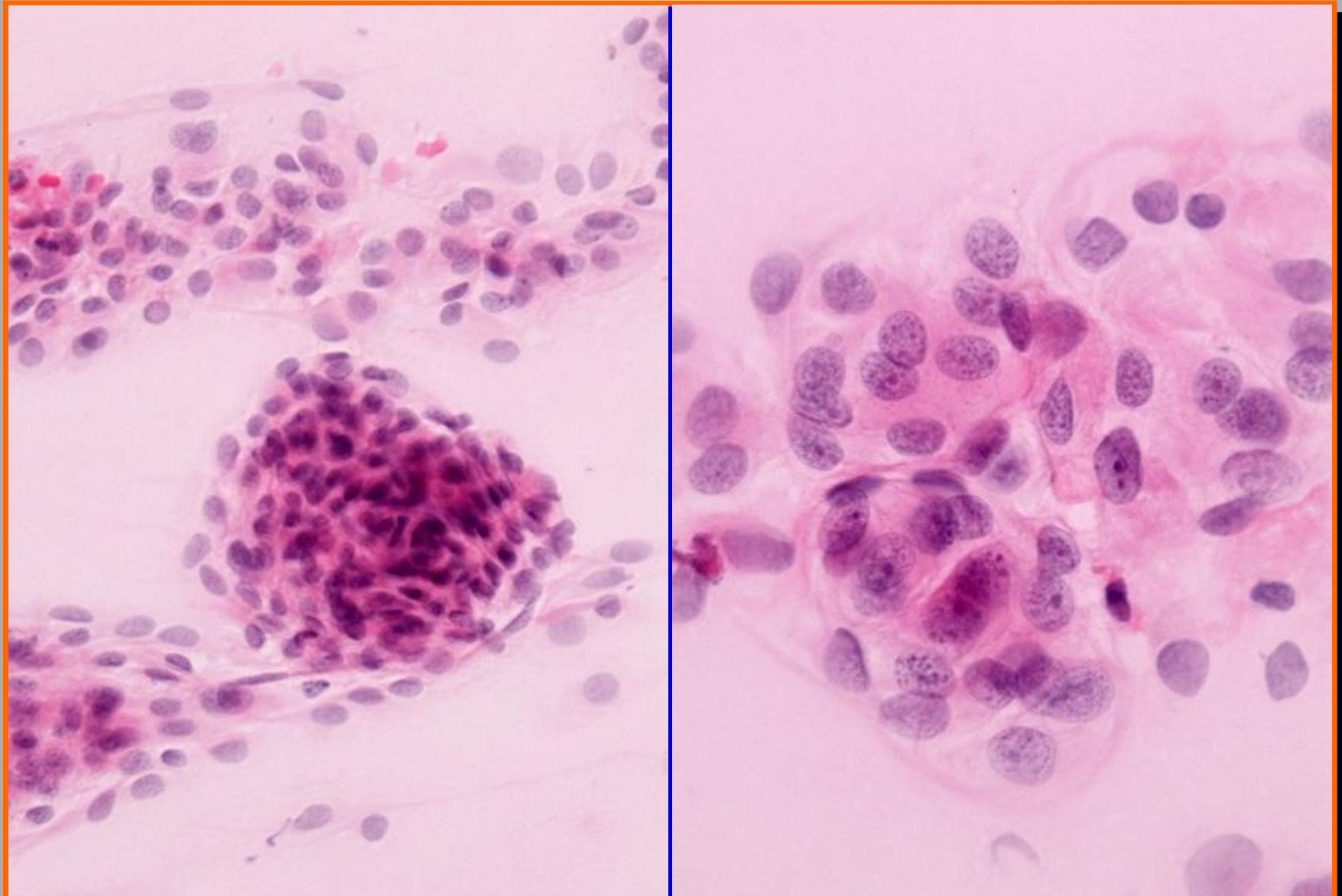
frozen section Lymphoma smear preparation



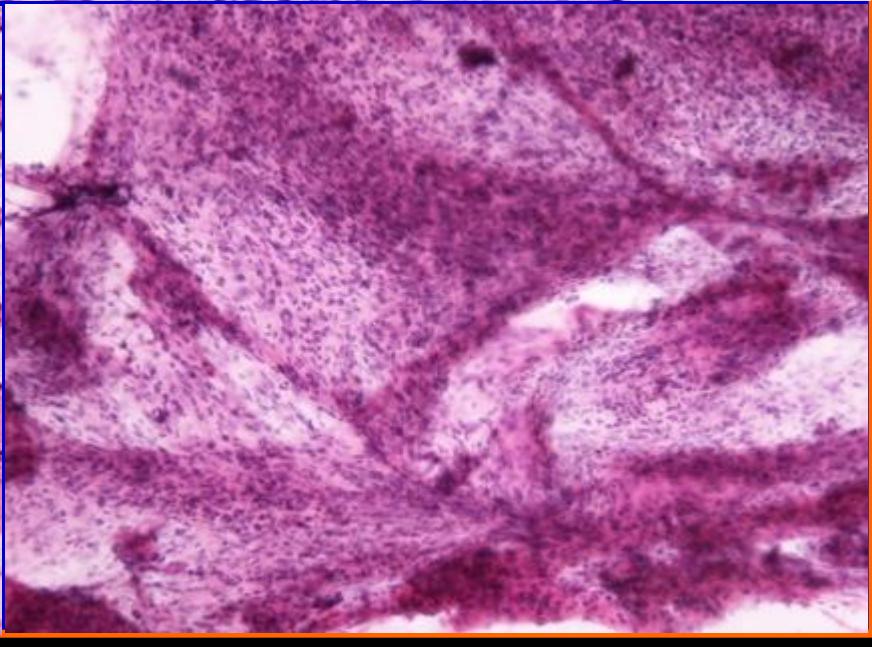
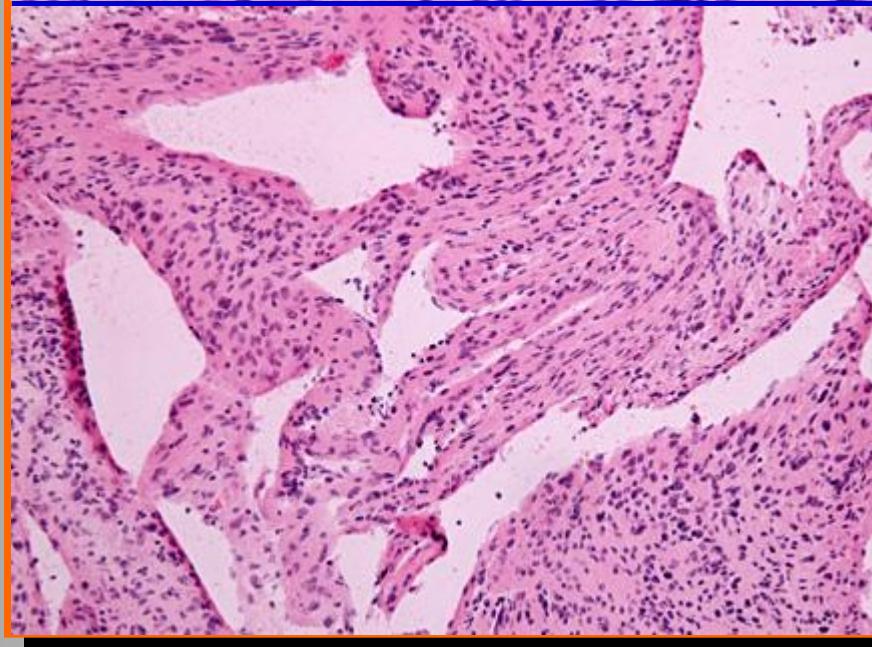
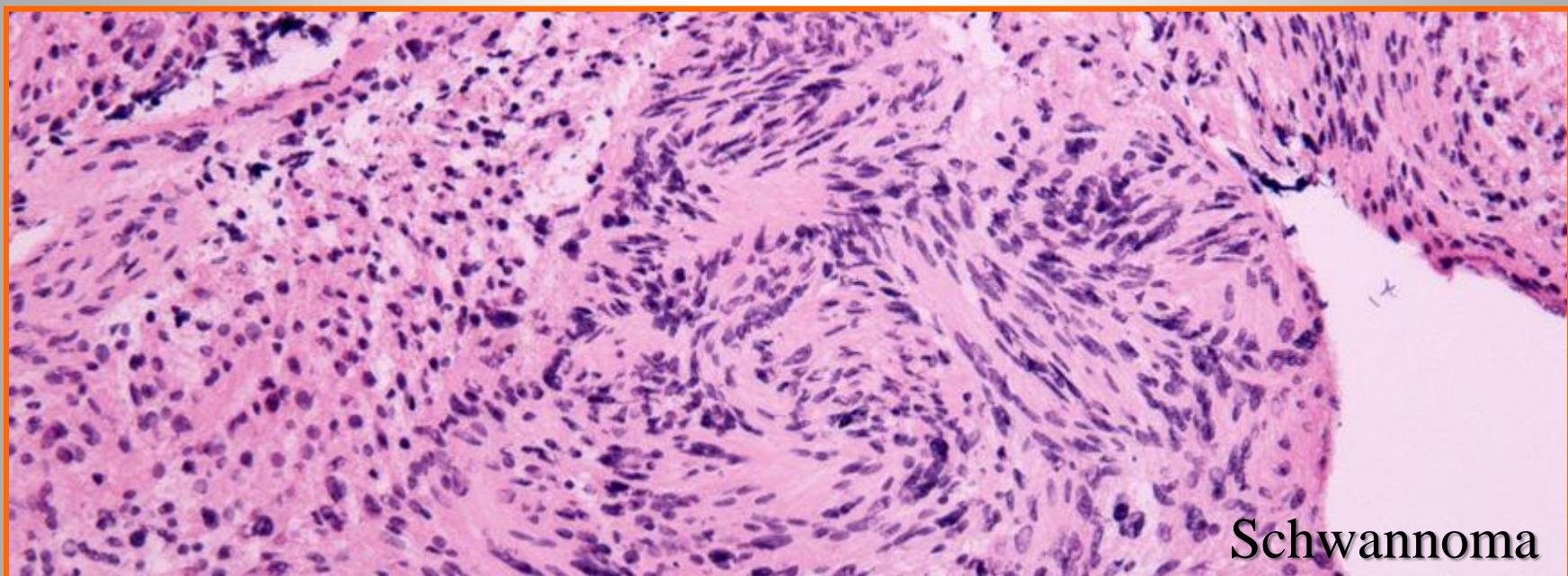
Lymphoma, lymphoglandular body

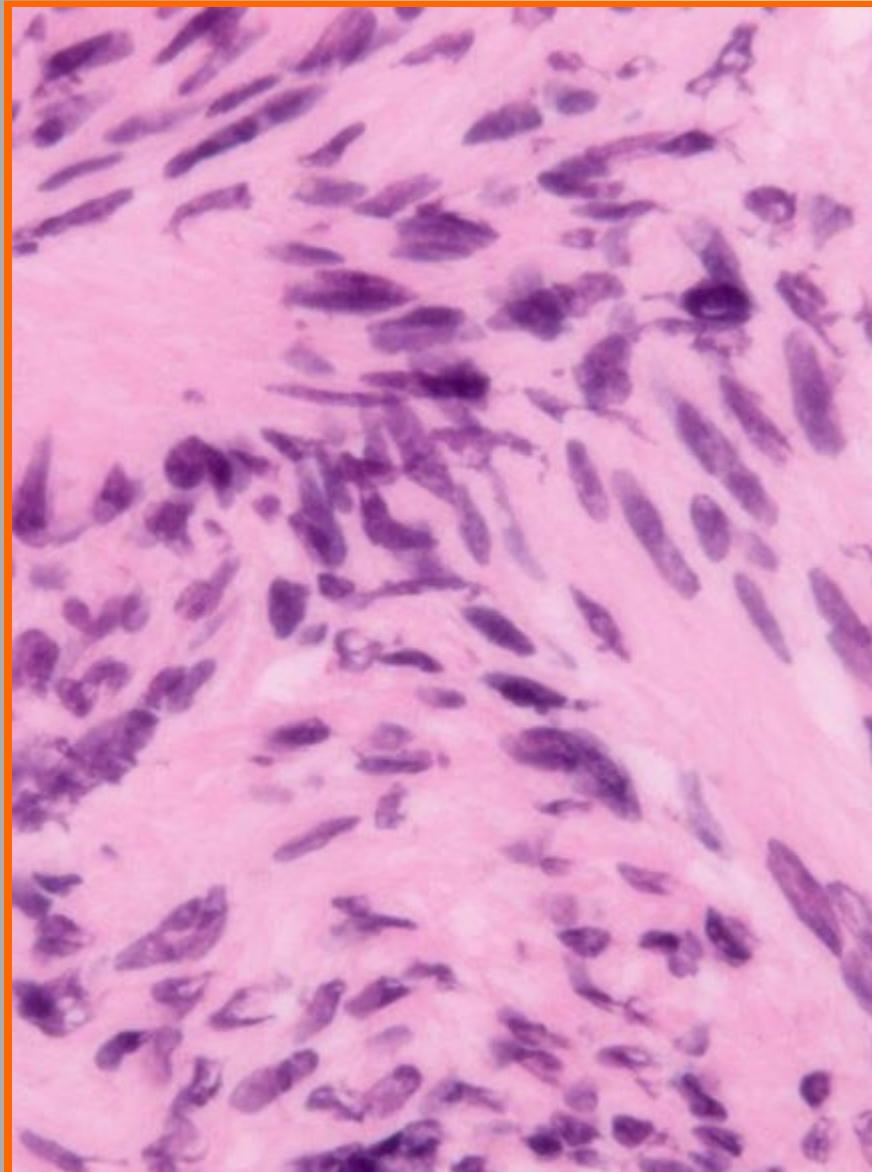


Meningioma, frozen section



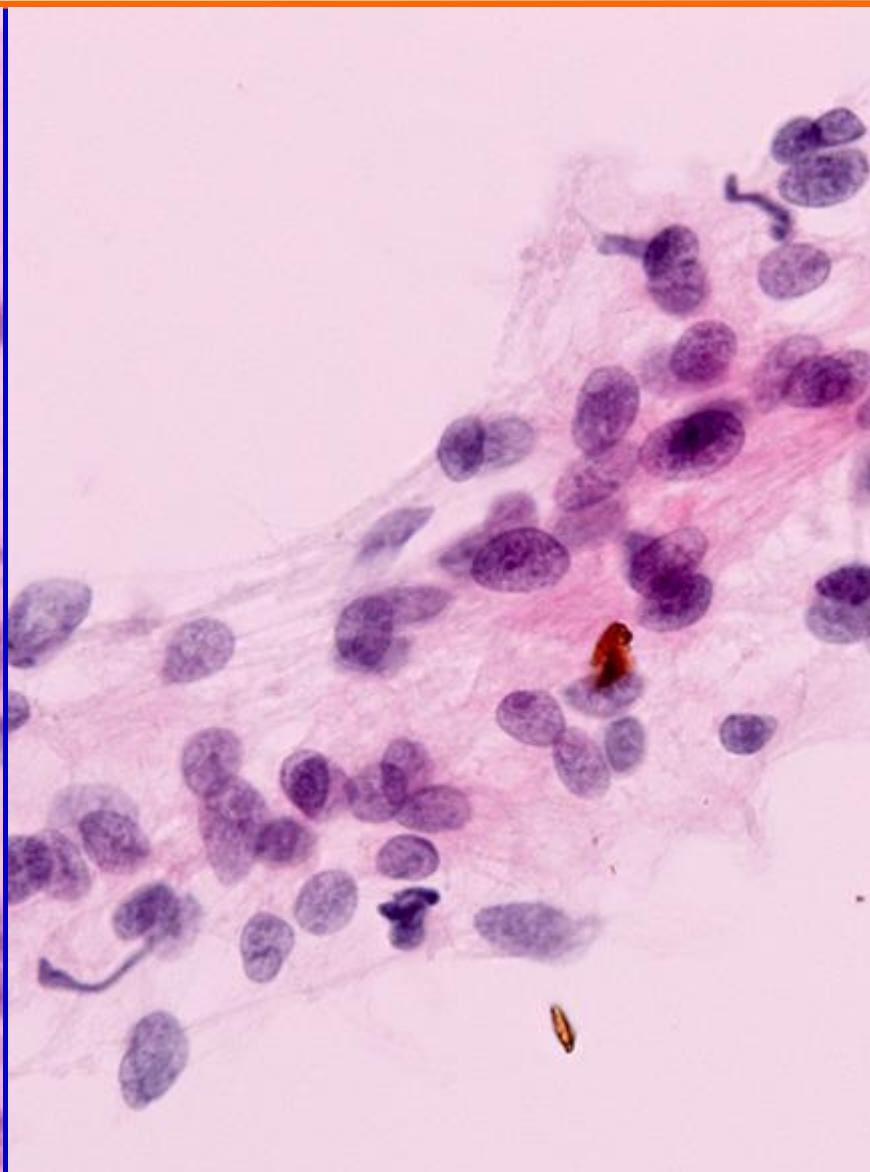
Meningioma, smear preparation





frozen section

Schwannoma



smear preparation

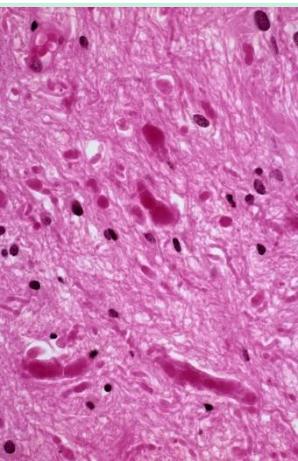
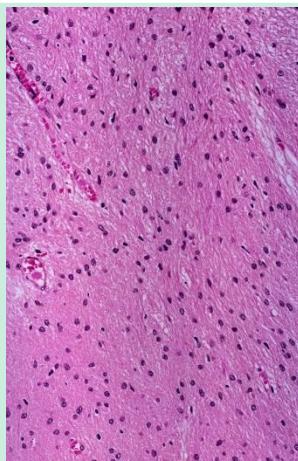
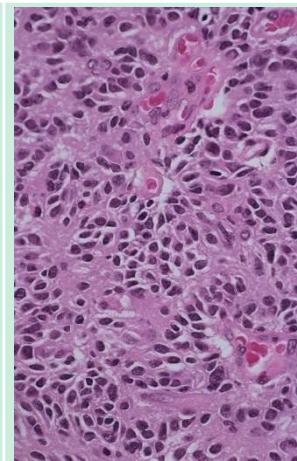
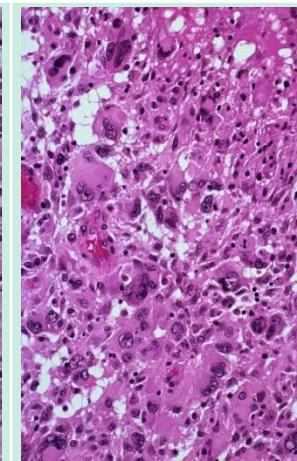
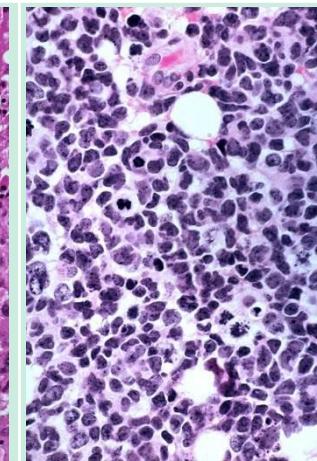
脳腫瘍の術中迅速診断

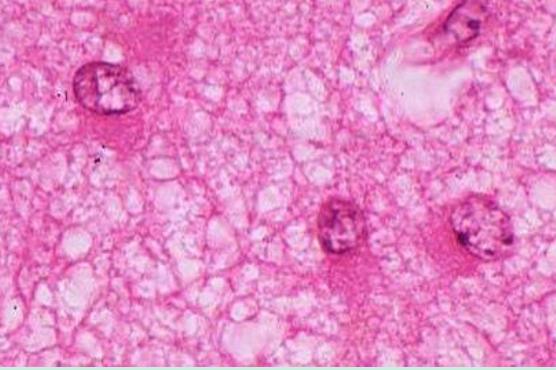
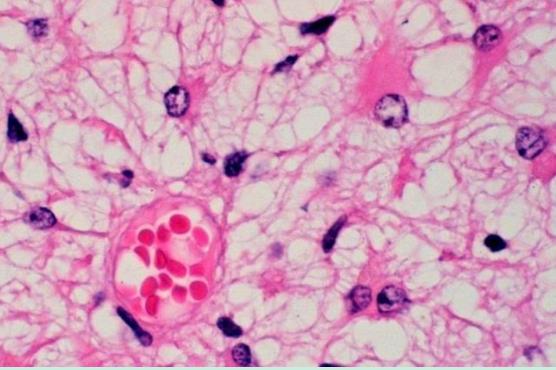
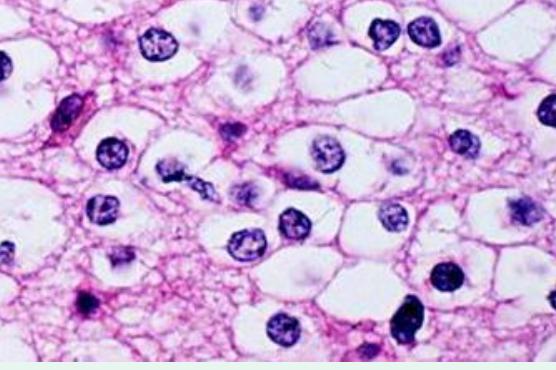
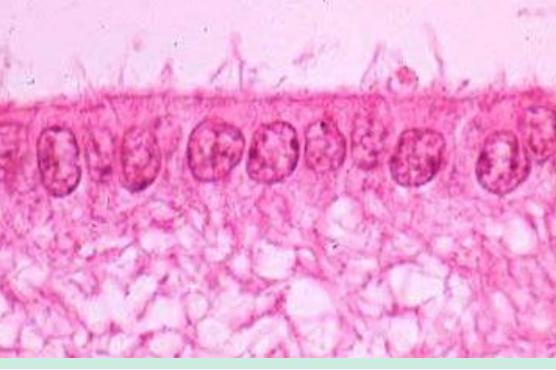
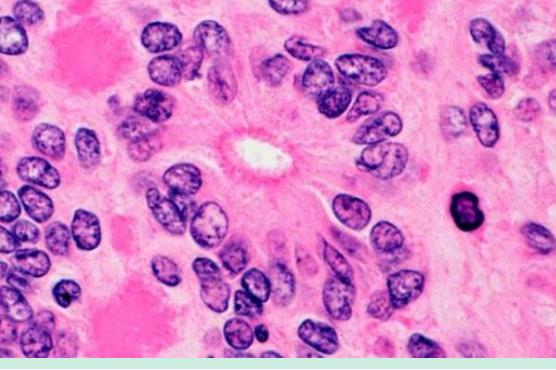
- 臨床情報、画像情報を吟味する。
- 凍結切片：細胞密度、組織構築に注目
- 塗沫標本：核と細胞質の微細な形態
- 診断時に術者と直接情報交換をする。
- 控えめな診断に留めてかまわない。
 - “low grade glioma”
 - “high grade glioma”
 - “meningioma”

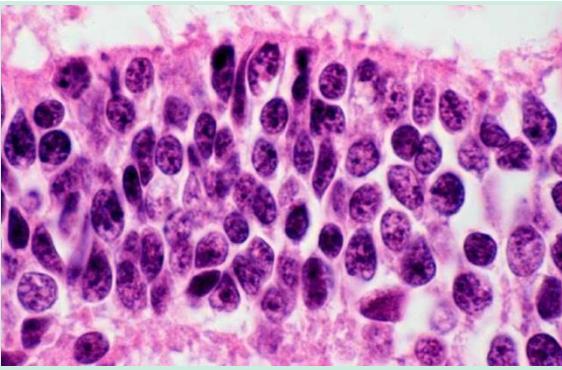
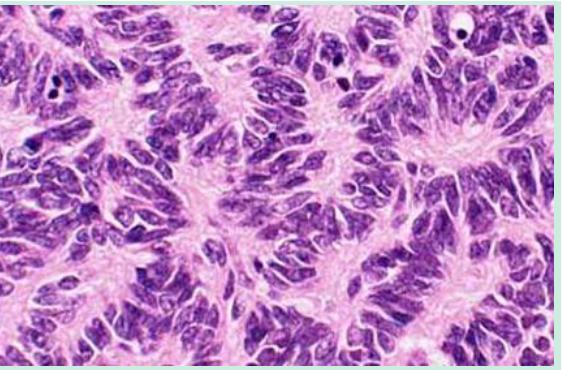
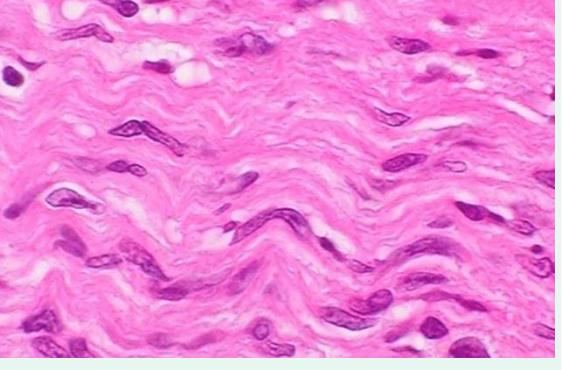
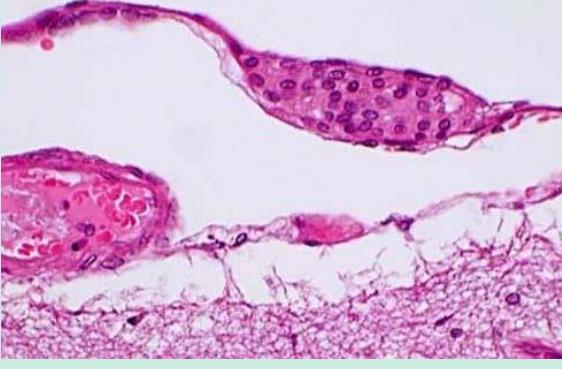
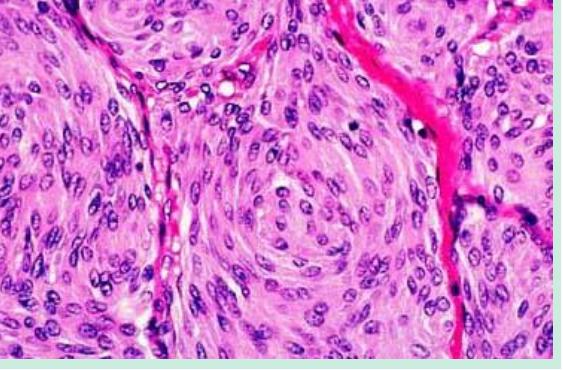
生検・手術例の診断

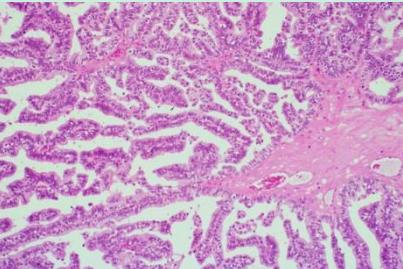
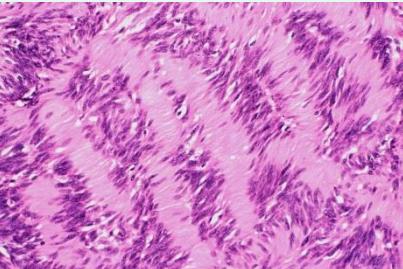
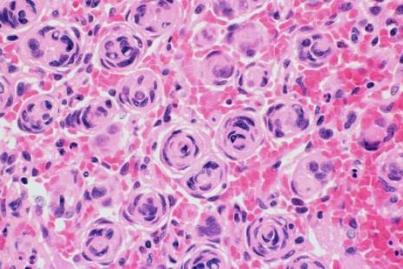
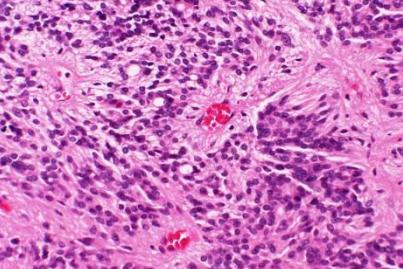
- 光顕像が診断の基礎
 - 細胞密度、細胞形態、組織パターン
- 免疫染色は診断精度を高める。
 - 細胞特異抗原、遺伝子産物、増殖能
- 電顕と遺伝子解析はオプショナル
 - 新規腫瘍型の確立には電顕が必須
 - 遺伝子解析には生材料の凍結保存を

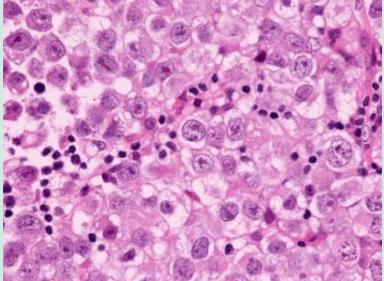
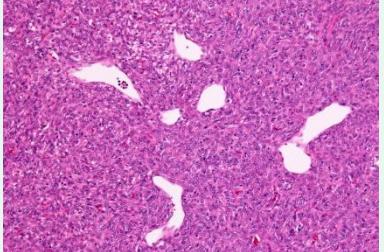
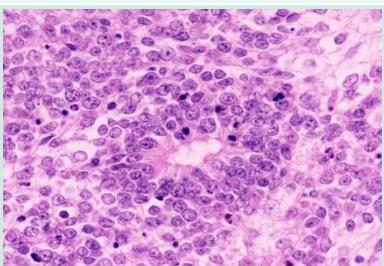
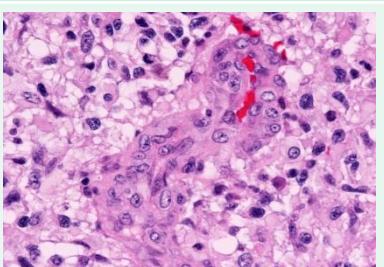
Cellularity

Low	Moderate	Slightly high	High	Very high
				
Pilocytic a. Subependym.	Diffuse astro. Gangliogliom. DNT Schwannoma	Oligodendrogl. Ependymoma Meningioma Pituitary aden.	Glioblastoma Anaplastic oligodendrogl. Anaplastic ependymoma	Medulloblast. PNET Lymphoma Germinoma

Normal cell	Tumor cell	Diagnosis
		<p>Diffuse astrocytoma Anaplastic astrocytoma Pilocytic astrocytoma Subependymal giant cell astrocytoma Glioblastoma</p>
		<p>Oligodendroglioma Anaplastic oligodendrogloma Oligoastrocytoma Anaplastic oligoastrocytoma</p>
		<p>Ependymoma Anaplastic ependymoma</p>

Normal Cell	Tumor cell	Diagnosis
		<p>Medulloblastoma PNET Atypical teratoid/rhabdoid tumor CNS neuroblastoma Ependymoblastoma</p>
		<p>Schwannoma MPNST Neurofibroma</p>
		<p>Meningioma Atypical meningioma Anaplastic meningioma</p>

Arrangement	Name	Tumor
	Papillary structure	Choroid plexus papilloma Papillary ependymoma Myxopapillary ependymoma Papillary meningioma
	Nuclear palisading	Schwannoma
	Whorl formation	Meningioma
	Perivascular pseudo-rosette	Ependymoma Astroblastoma Astrocytoma Glioblastoma

Arrangement	Name	Tumor
	Two cell pattern	Germinoma
	Stag horn vessels	Hemangiopericytoma Solitary fibrous tumor Mesenchymal chondrosarcoma
	Ependymoblastic rosette	Ependymoblastoma ETANTR Medulloepithelioma
	Microvascular proliferation	Glioblastoma Anaplastic oligodendrogloma Anaplastic ependymoma

脳腫瘍の光顕像

- 細胞密度: 5段階 → およその悪性度
- 細胞形態: 類似性 → 組織由来を示唆
 同上 : 核異型 → gradeを示唆
- 組織構築: 多種類ある
 - a. 腫瘍型に特異的なもの
 - b. いくつかの腫瘍型に出現するもの
 - c. 腫瘍の悪性度を示唆するもの

脳腫瘍と免疫染色

- 神経組織特異抗原の存在
 - 免疫組織化学的方法の進歩
 - 抗体作製：モノクローナル抗体法
 - 脳腫瘍診断への応用
 - 細胞由来の同定
 - 細胞増殖能の評価
 - 遺伝子異常の推定
- 細胞特異マーカー
- 増殖マーカー
- 異常な遺伝子産物

診断に頻用する抗原

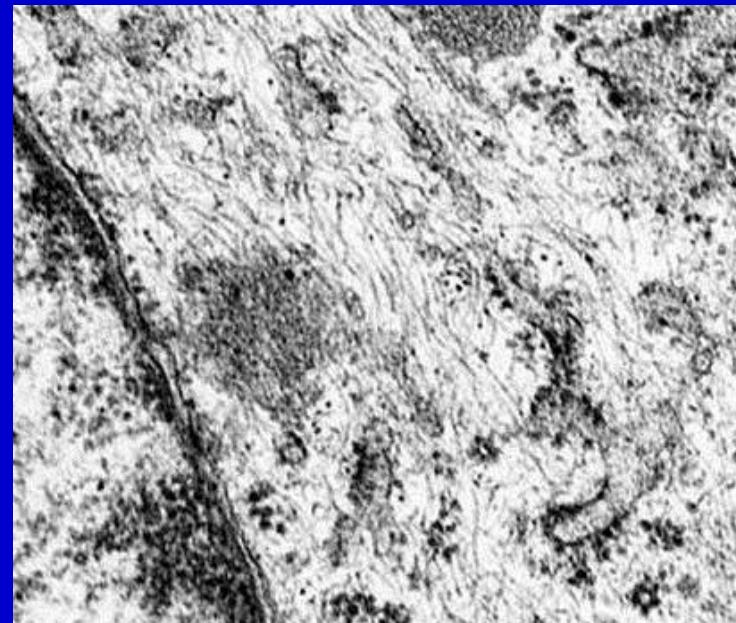
- Glioma: GFAP, S-100P, Olig2, nestin, mIDH1
- Neuronal, glioneuronal tumor: NFP, synaptophysin, NeuN, chromogranin A, Olig2
- Embryonal tumor: INI-1
- Nerve tumors: S-100P, Schwann/2E, vimentin
- Meningioma: EMA, vimentin, D2-40, CD34
- Germ cell tumor: PLAP, c-Kit, Oct4, D2-40
- Lymphoma: CD3, CD5, CD10, CD20, bcl-2
- Hemangiopericytoma, SFT: CD34
- Proliferating cells: Ki-67

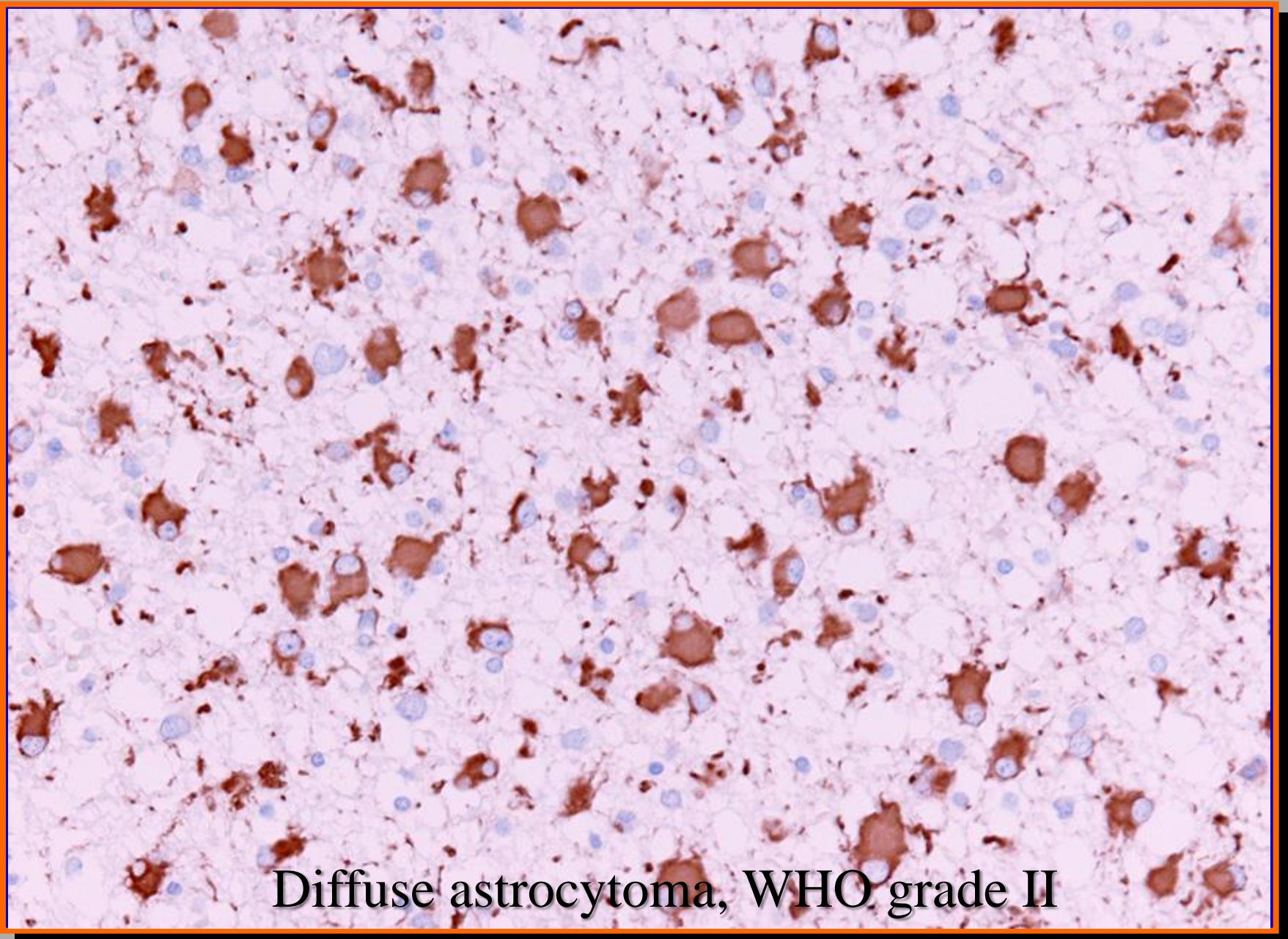
診断に頻用する抗原

- Glioma: GFAP, S-100P, Olig2, nestin, mIDH1
- Neuronal, glioneuronal tumor: NFP, synaptophysin, NeuN, chromogranin A, Olig2
- Embryonal tumor: INI-1
- Nerve tumors: S-100P, Schwann/2E, vimentin
- Meningioma: EMA, vimentin, D2-40, CD34
- Germ cell tumor: PLAP, c-Kit, Oct4, D2-40
- Lymphoma: CD3, CD5, CD10, CD20, bcl-2
- Hemangiopericytoma, SFT: CD34
- Proliferating cells: Ki-67

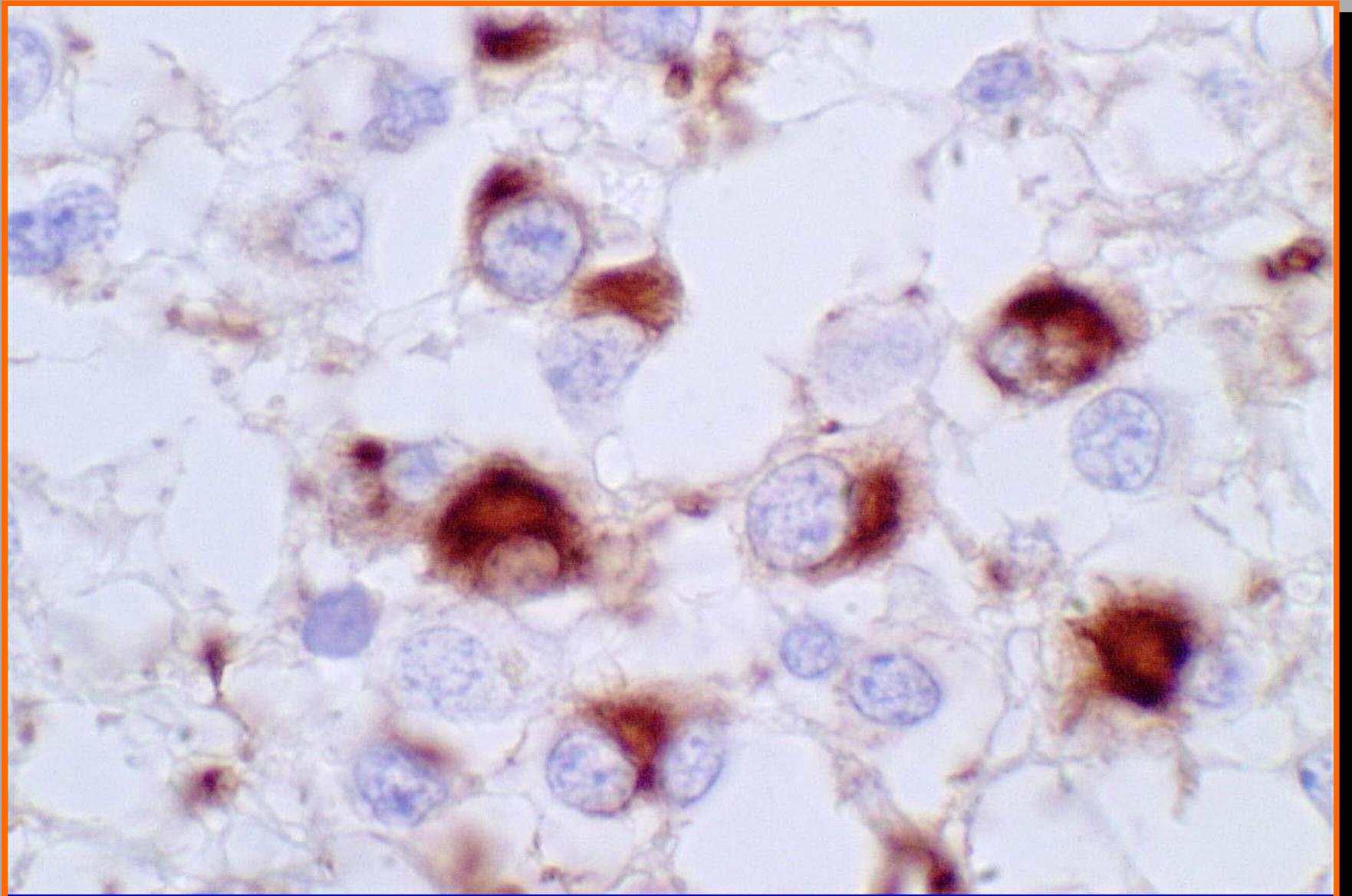
GFAP

- Glial fibrillary acidic protein
- グリア細線維を構成する49kDaの蛋白
- 局在: astrocyte
- 腫瘍: astrocytoma,
oligodendrogloma,
ependymoma, etc.
- * pleomorphic adenoma

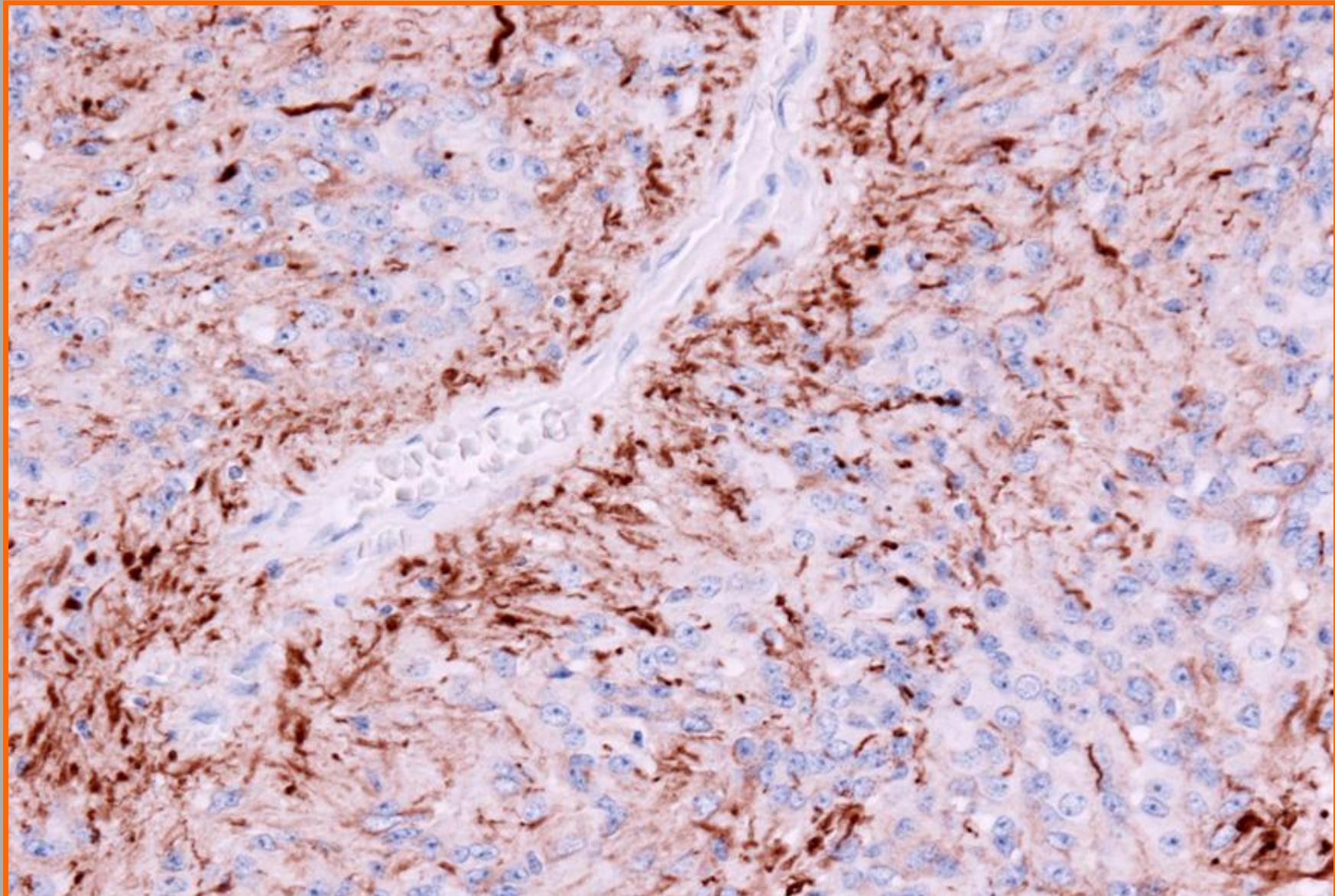




Diffuse astrocytoma, WHO grade II



Oligodendrogloma, WHO grade II

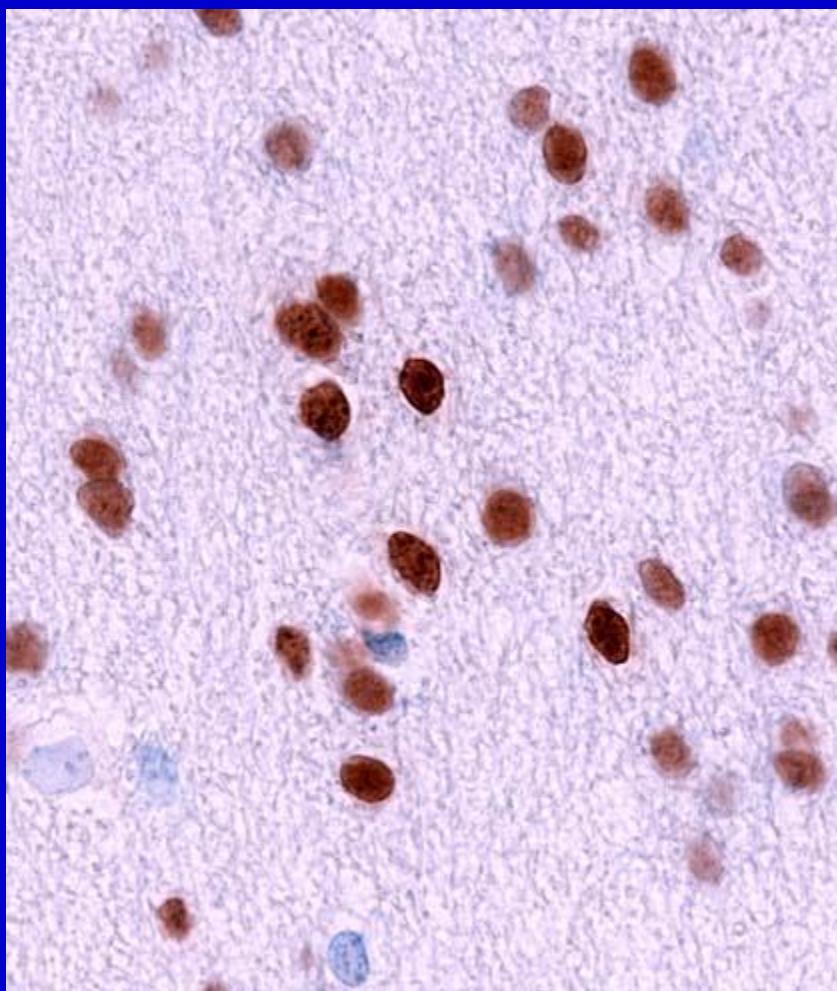


Ependymoma, WHO grade II

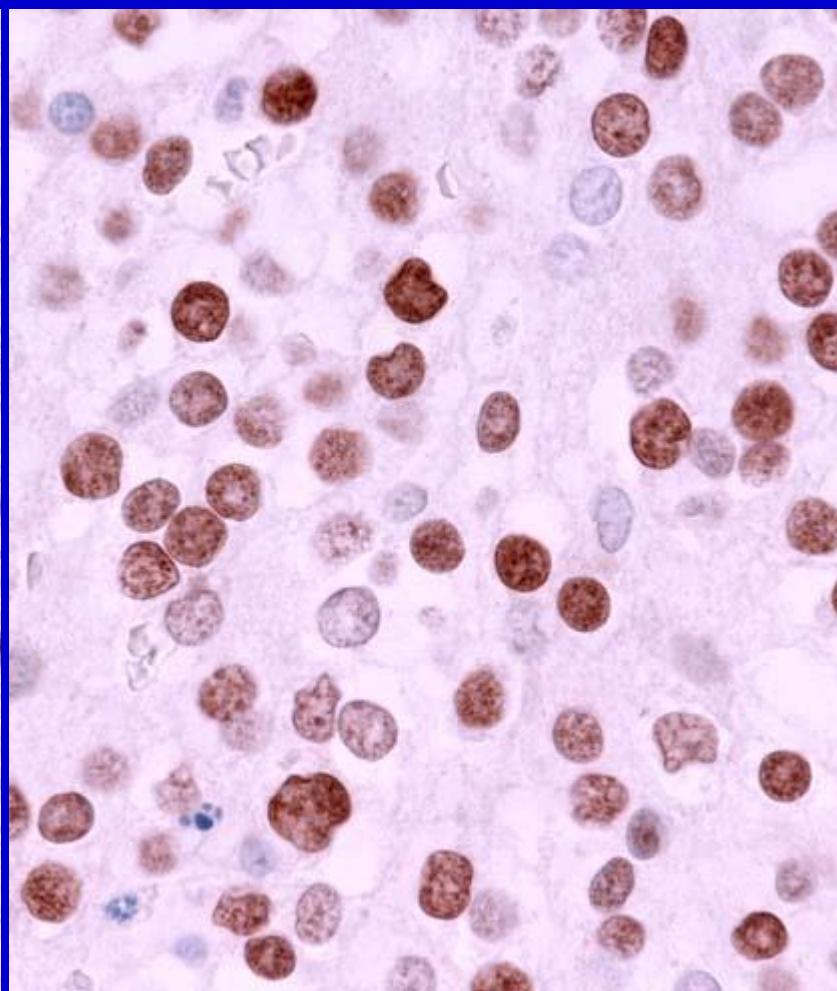
Olig2

- basic helix-loop-helix 転写因子, 32 kDa
- 胎生期の神経組織に発現: 乏突起膠細胞と神経細胞の発生・分化に関与している。
- 正常: oligodendroglia
- 腫瘍: oligodendroglioma, oligoastrocytoma, diffuse astrocytoma, pilocytic astrocytoma, glioneuronal tumors

Olig2



Normal white matter



Oligodendrogloma

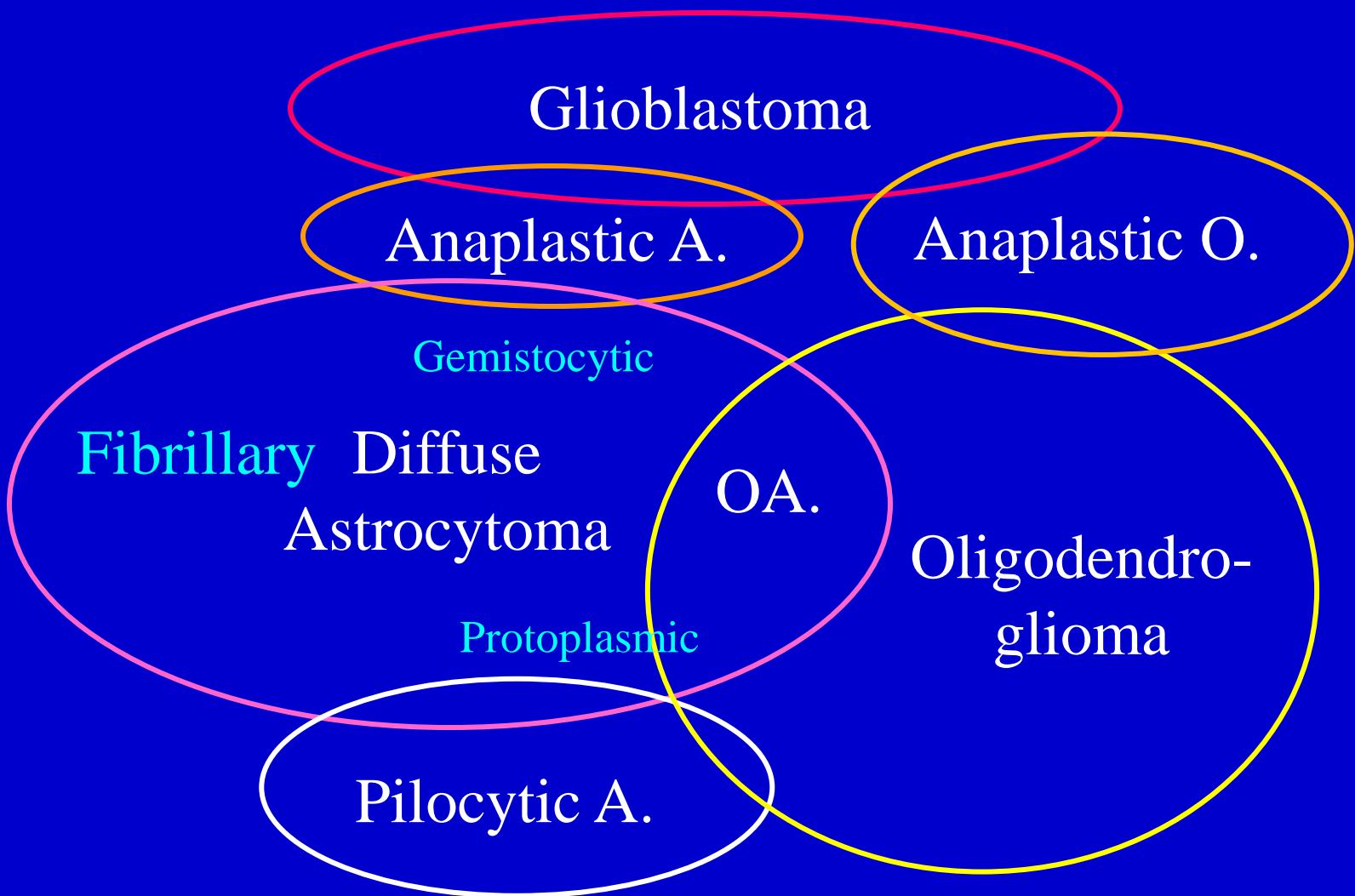
Olig2 expression in human brain tumors

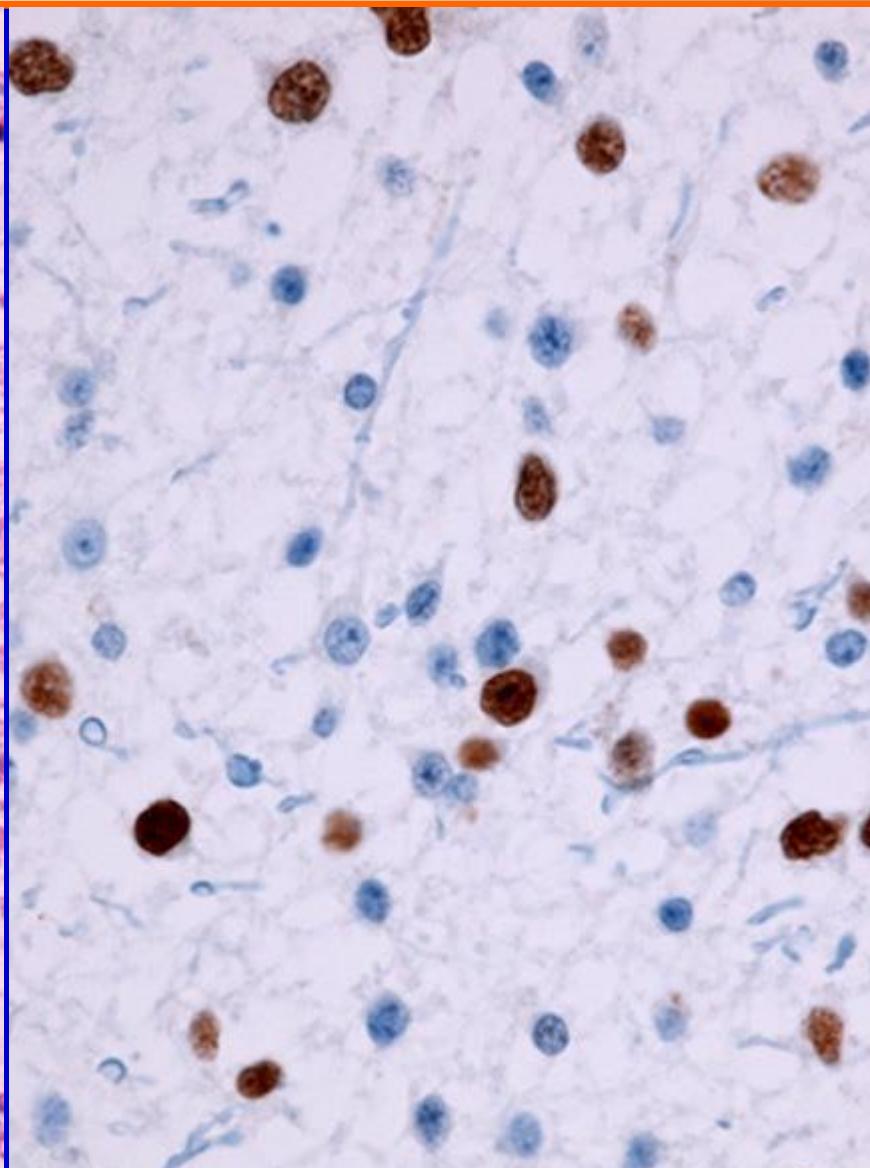
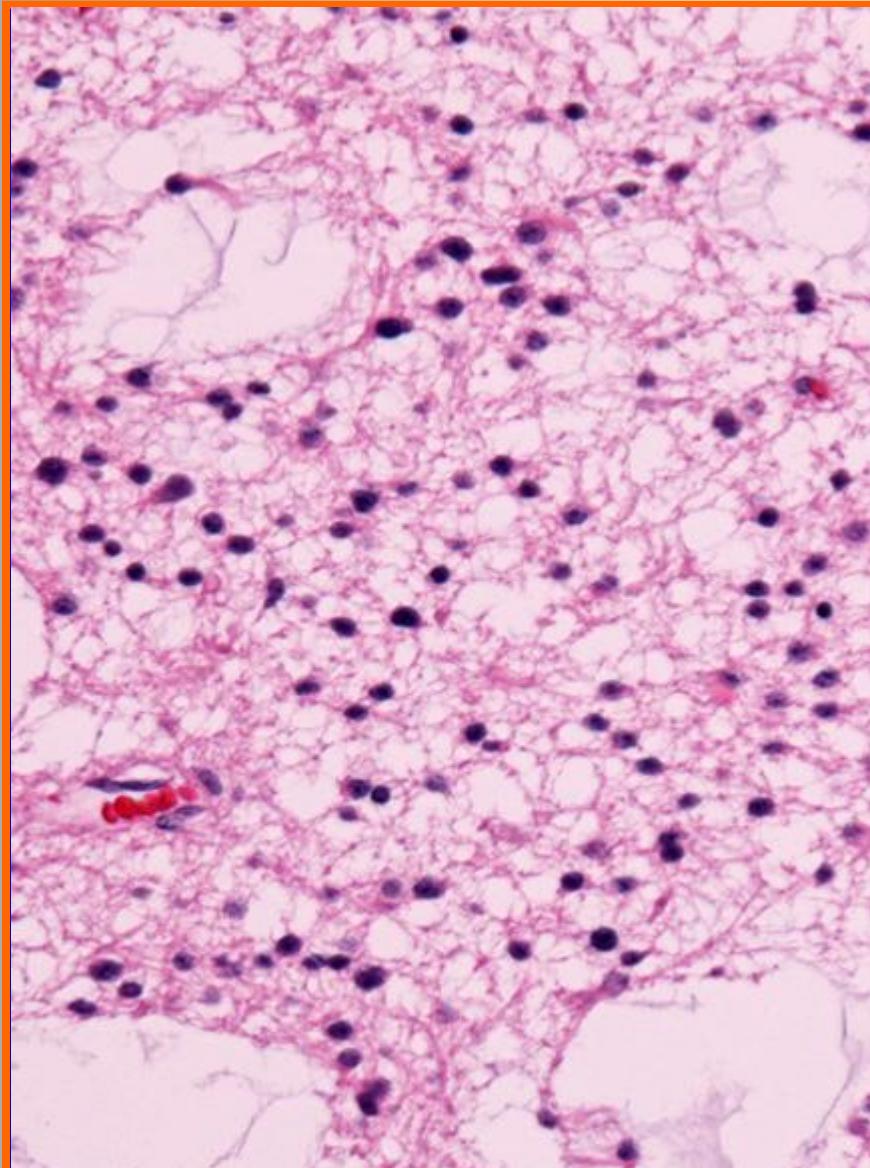
Histology	Total	-	+	++	+++

oligodendroglial tumors	40	0	8	13	19
oligodendroglioma	16	0	3	6	7
oligoastrocytoma	3	0	0	1	2
anaplastic oligo.	16	0	3	5	8
anaplastic oligoastro.	5	0	2	1	2
astrocytic tumors	35	6	17	6	6
pilocytic astrocytoma	4	0	2	1	1
fibrillary astrocytoma	10	0	7	2	1
anaplastic astrocytoma	8	1	3	1	3
glioblastoma	13	5	5	2	1
central neurocytoma	6	6	0	0	0
schwannoma	11	11	0	0	0

Yokoo H. 2003

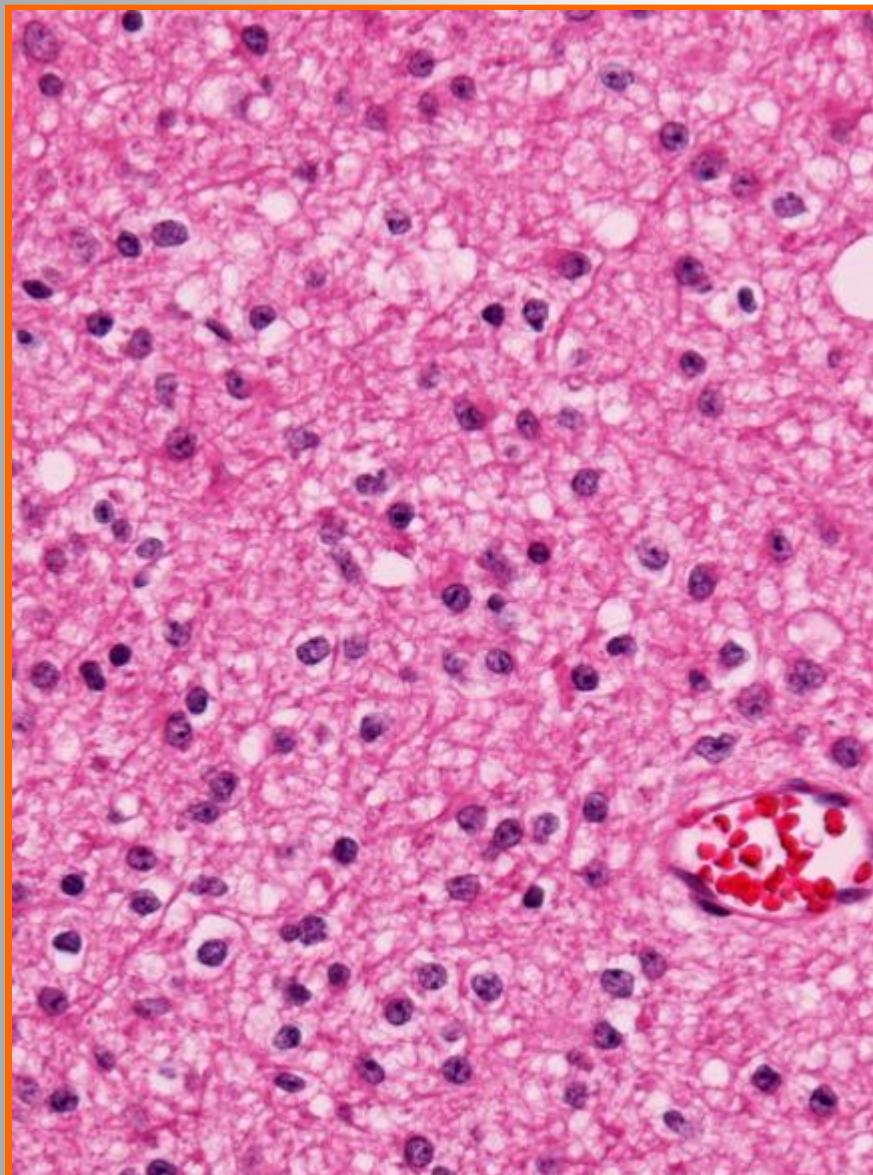
鑑別診斷: DA vs OA vs Oligo



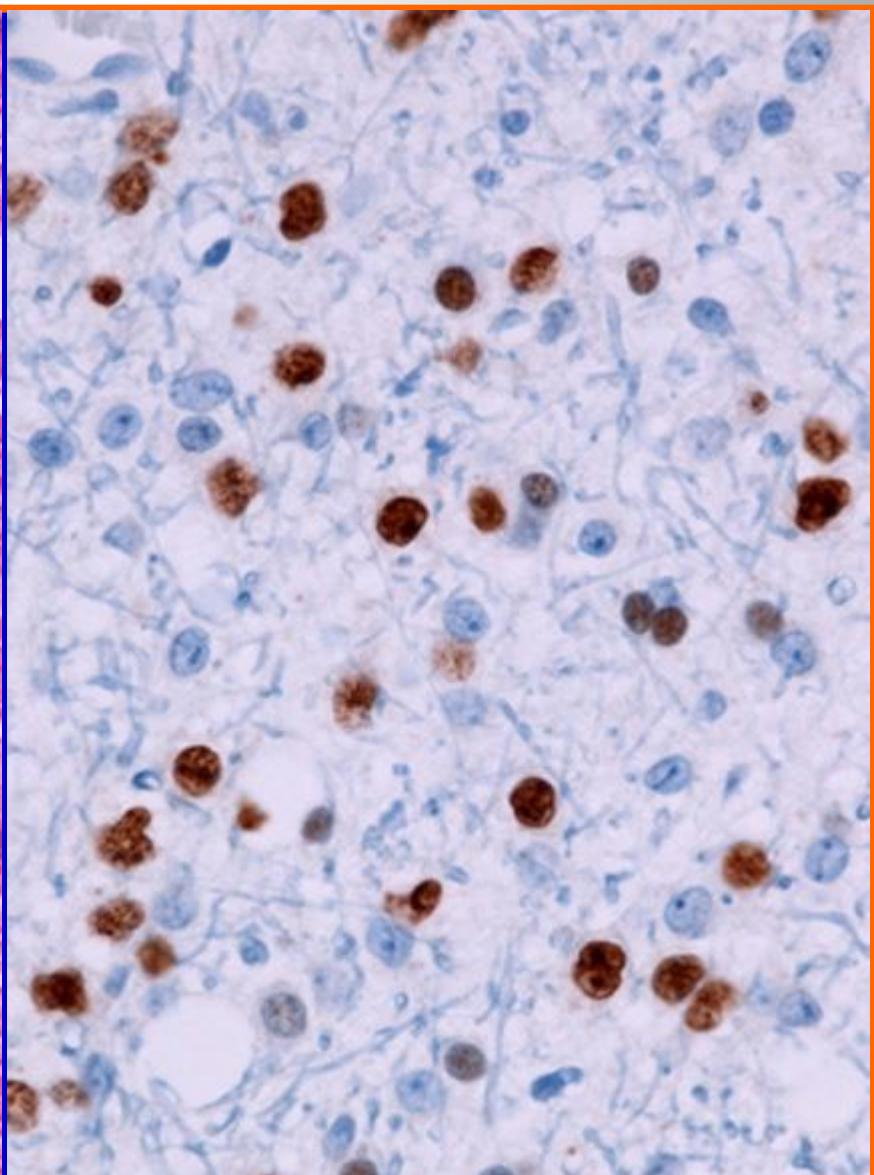


Astrocytoma

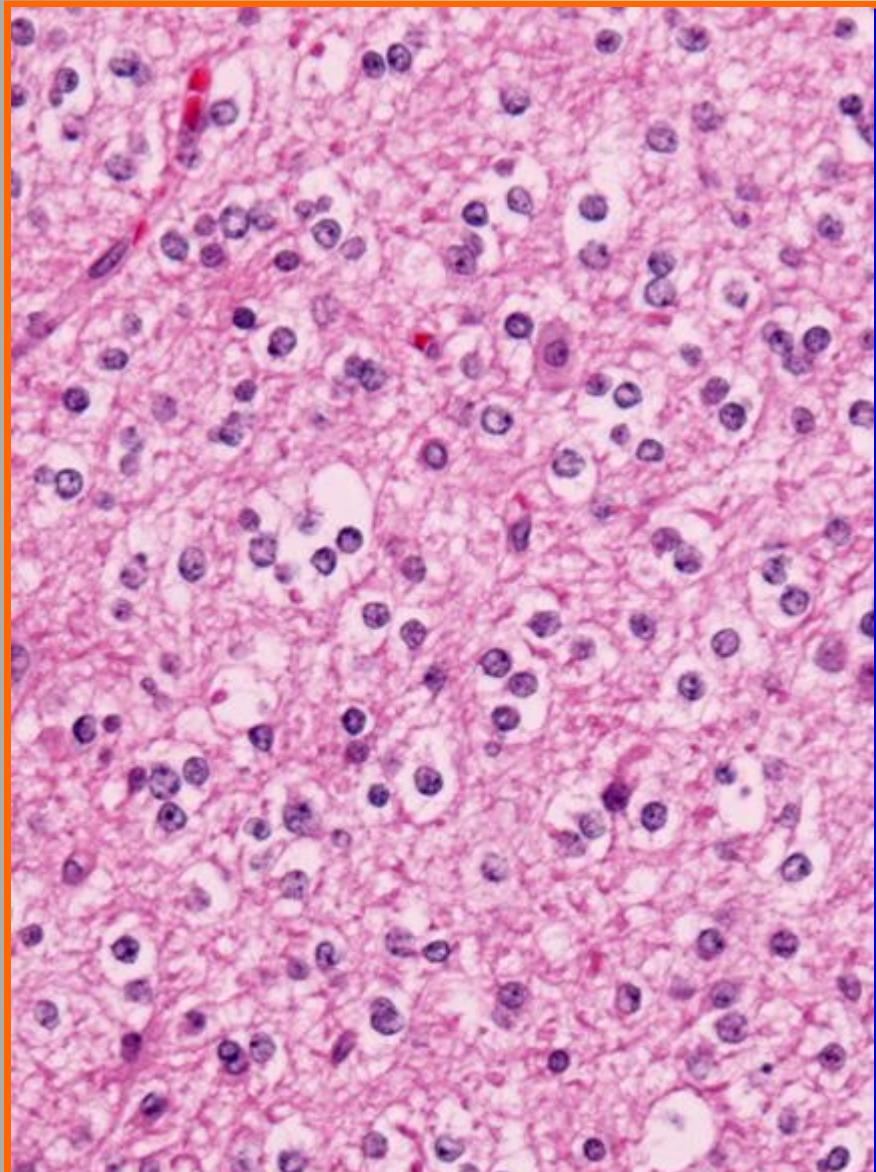
Olig2 SI= 48.5%



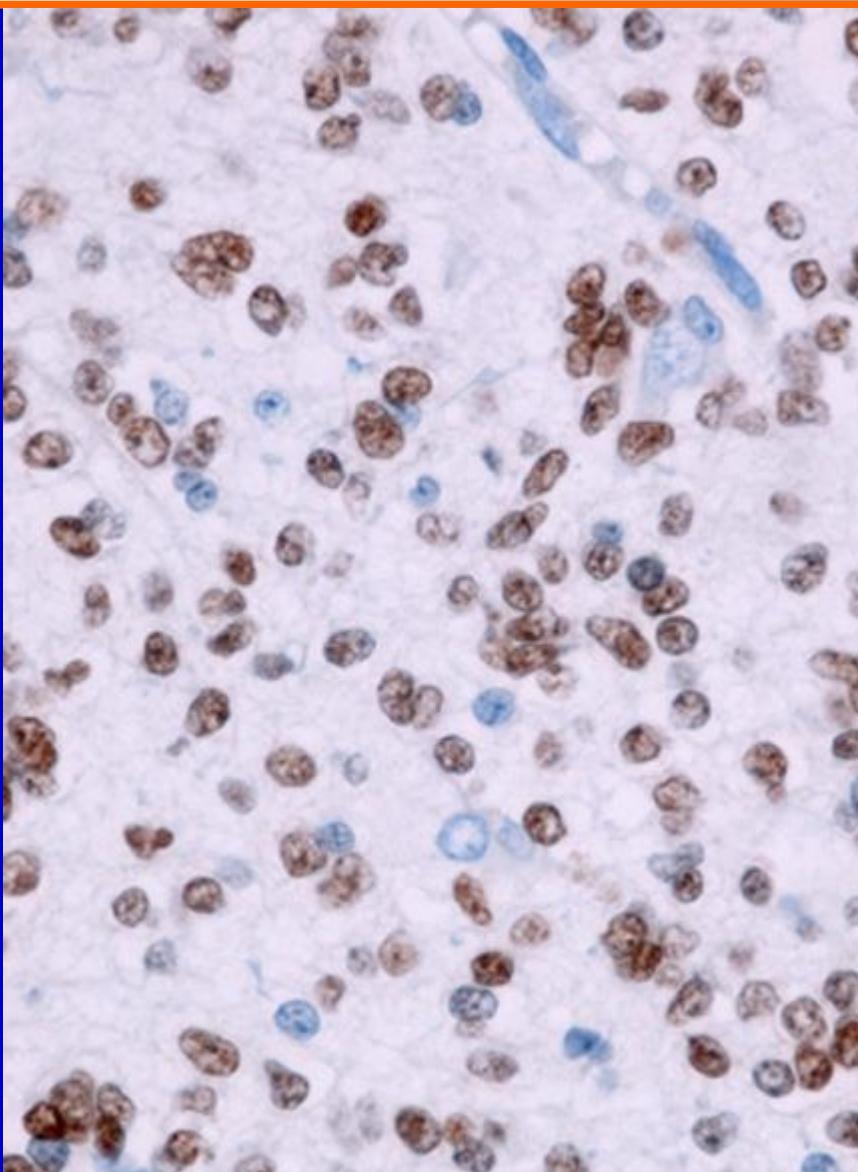
Oligoastrocytoma



Olig2 SI= 64.0%

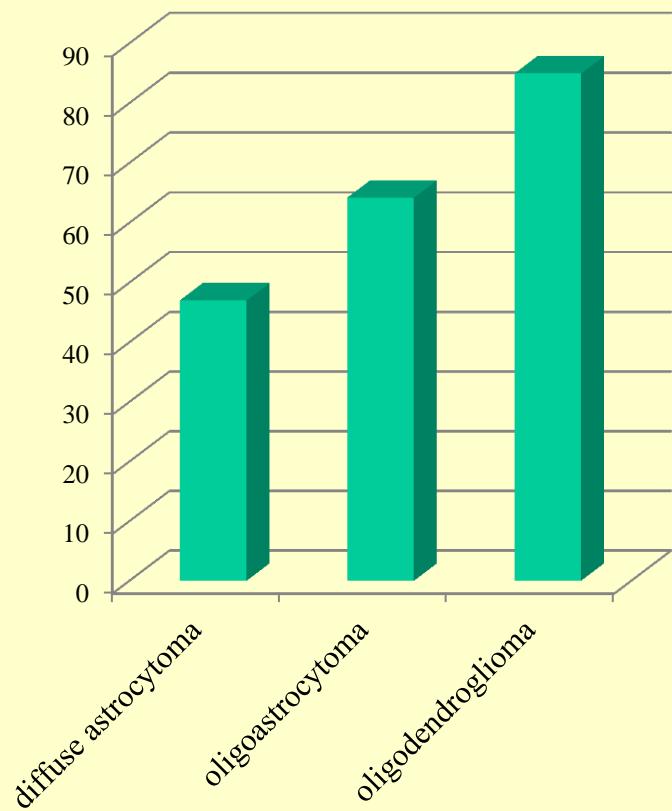


Oligodendrogloma

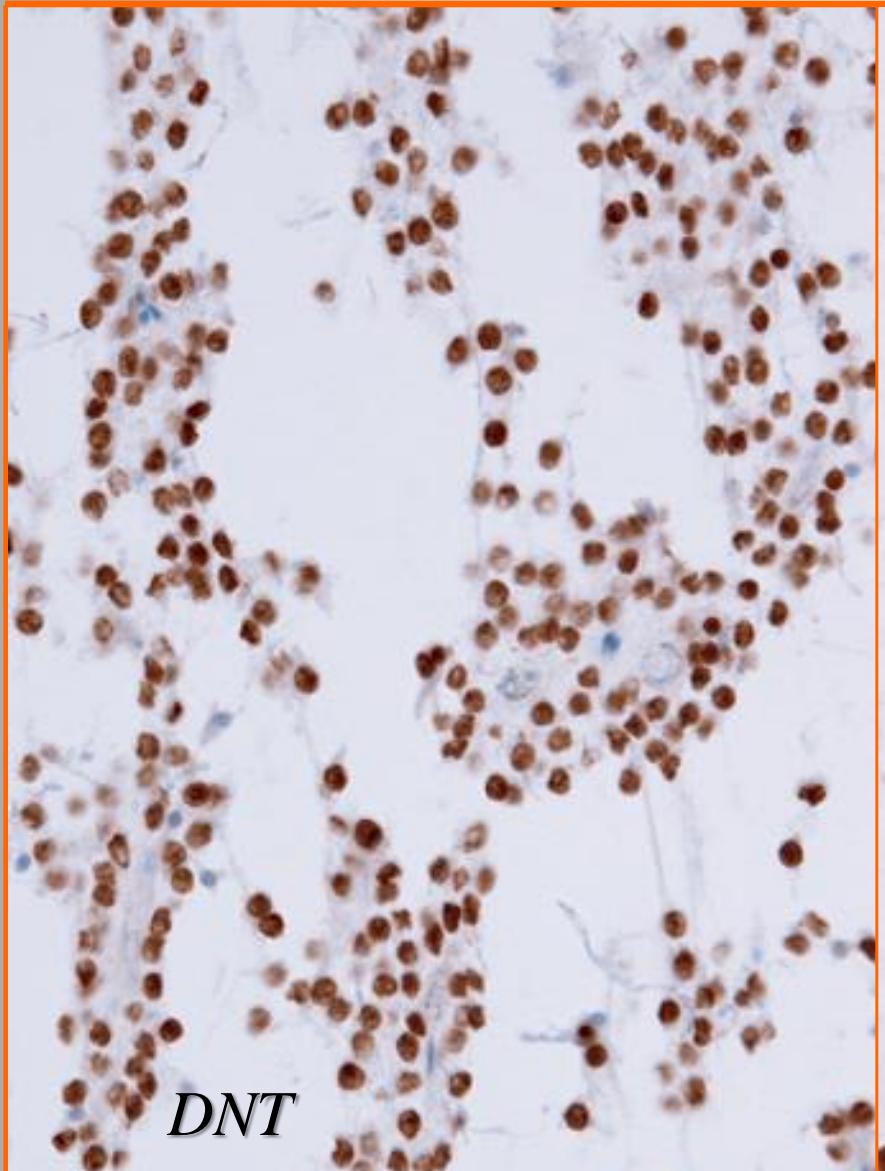


Olig2 SI= 94.3%

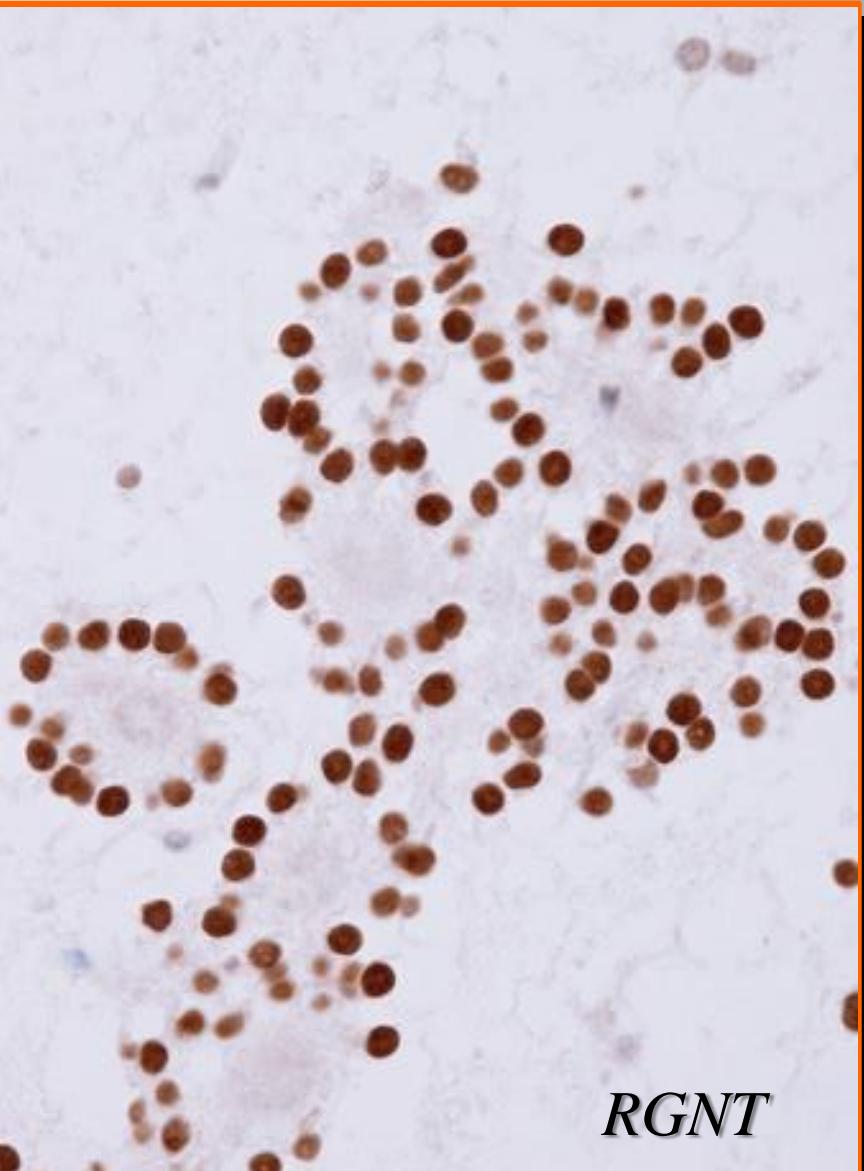
Olig2 Staining Index



Suzuki A, 2012



DNT



RGNT

Olig-2 in glioneuronal tumors

Matsumura N, 2012

Isocitrate dehydrogenase (IDH)

- Yan H, Parsons DW, et.al.

IDH1 and IDH2 mutations in gliomas.

N Engl J Med. 2009; 360(8):765-73.

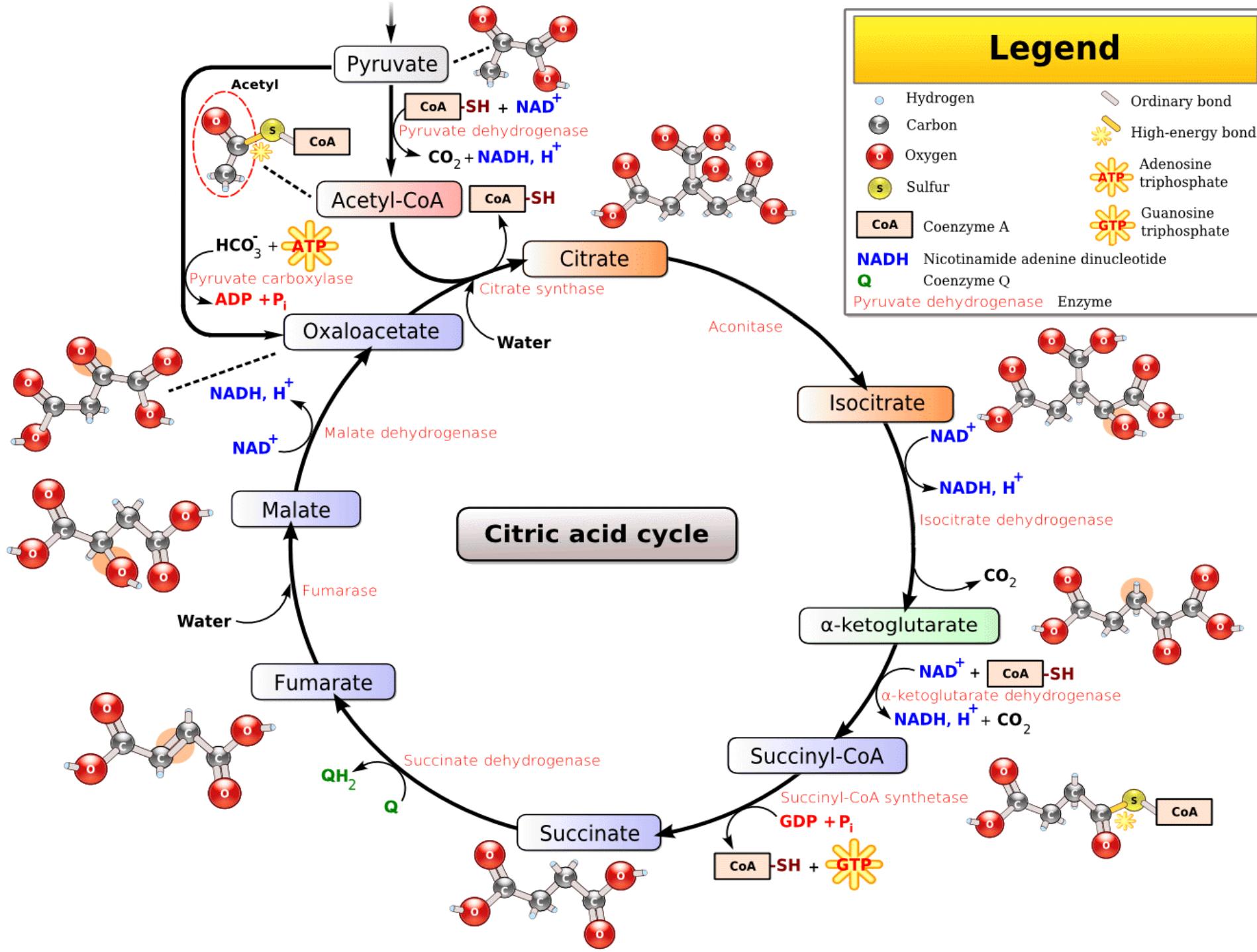
- Capper D, ,von Deimling A.

Characterization of R132H mutation-specific
IDH1 antibody binding in brain tumors.

Brain Pathol. 2010;20(1):245-54.

Legend

Hydrogen	Ordinary bond
Carbon	High-energy bond
Oxygen	Adenosine triphosphate
Sulfur	Guanosine triphosphate
CoA	Coenzyme A
NADH	Nicotinamide adenine dinucleotide
Q	Coenzyme Q
Pyruvate dehydrogenase	Enzyme



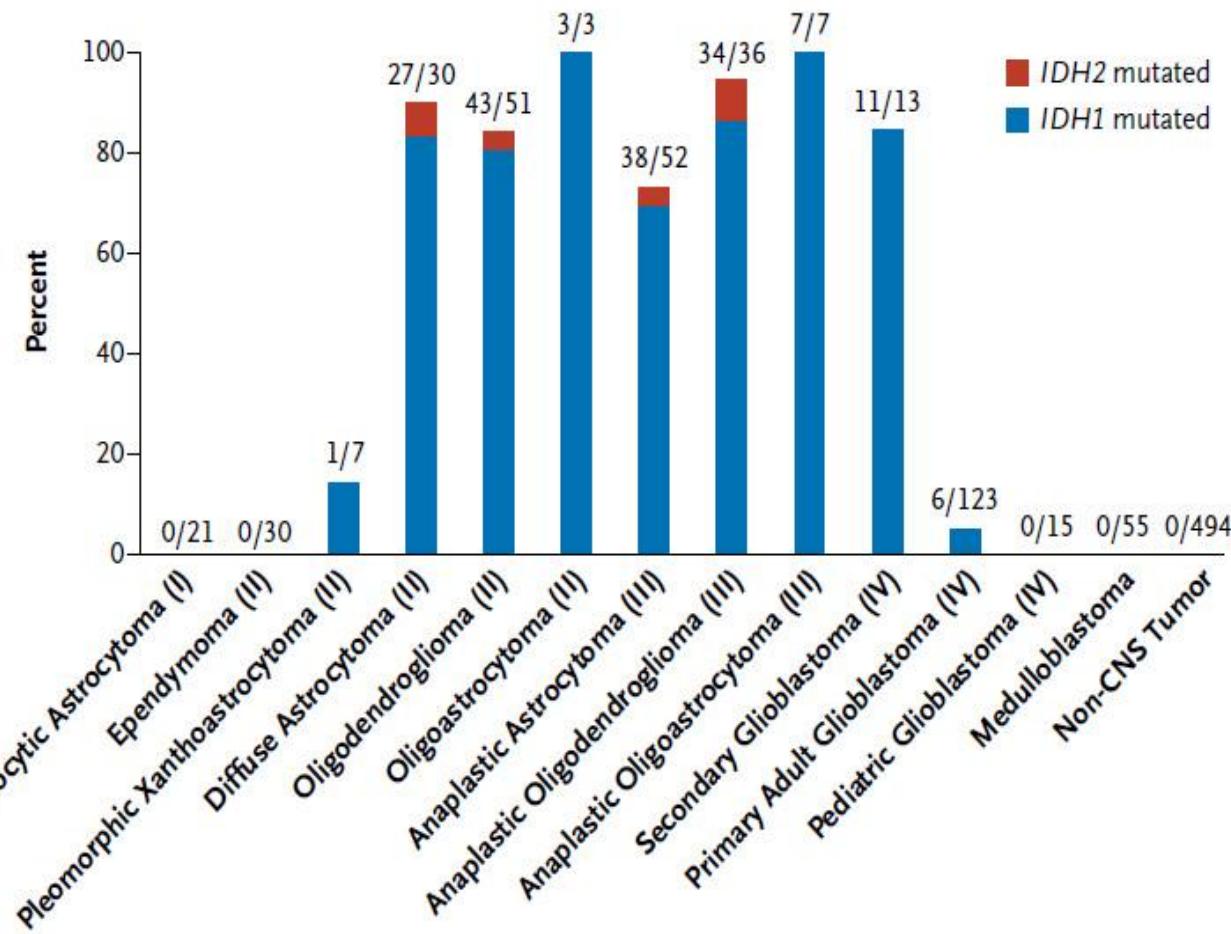
A Mutations

R172G GGG N=2
 R172M ATG N=3
 R172K AAG N=4

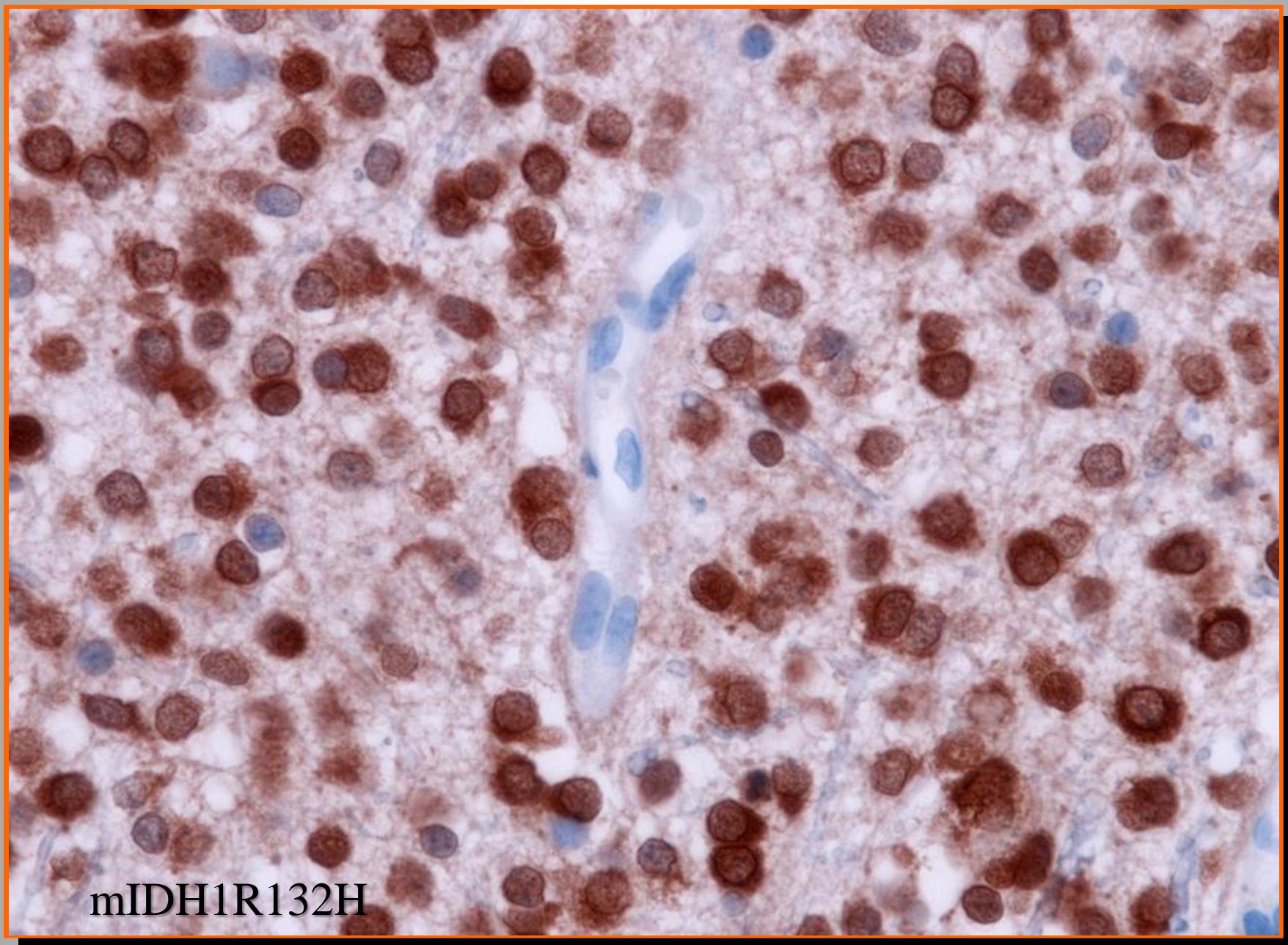
<i>IDH2</i>	ATT	GGC	AGG	CAC	GCC
	I ¹⁷⁰	G ¹⁷¹	R ¹⁷²	H ¹⁷³	A ¹⁷⁴

<i>IDH1</i>	I ¹³⁰	G ¹³¹	R ¹³²	H ¹³³	A ¹³⁴
	ATA	GGT	CGT	CAT	GCT

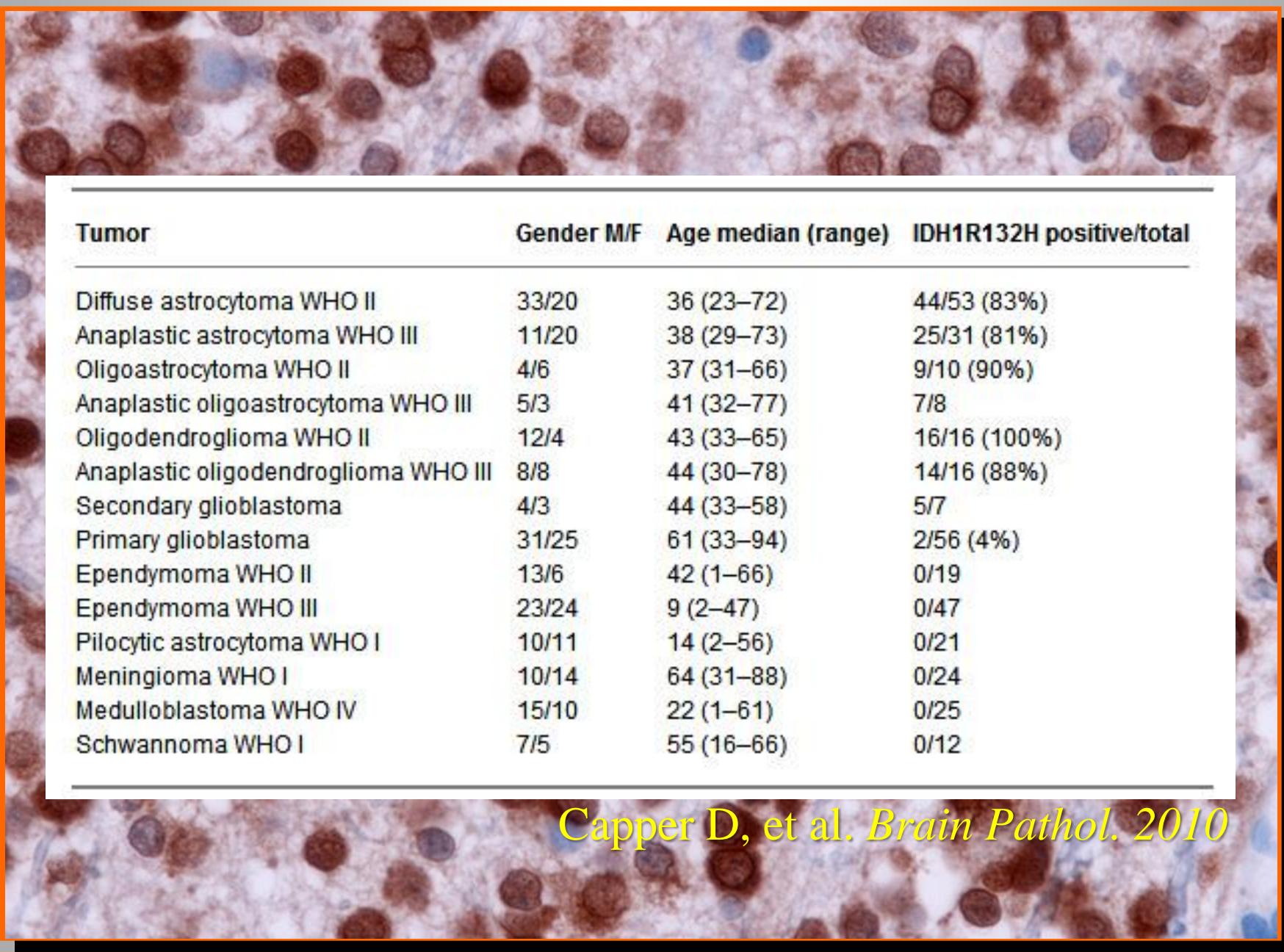
R132H CAT N=142
 R132C TGT N=7
 R132L CTT N=7
 R132S AGT N=4
 R132G GGT N=1

B Frequency of Mutations**Figure 1.** *IDH1* and *IDH2* Mutations in Human Gliomas.

Panel A shows mutations at codon R132 in *IDH1* and R172 in *IDH2* that were identified in human gliomas, along with the number of patients who carried each mutation. Codons 130 to 134 of *IDH1* and 170 to 174 of *IDH2* are shown. Panel B shows the number and frequency of *IDH1* and *IDH2* mutations in gliomas and other types of tumors. The roman numerals in parentheses are the tumor grades, according to histopathological and clinical criteria established by the World Health Organization. CNS denotes central nervous system.



mIDH1R132H



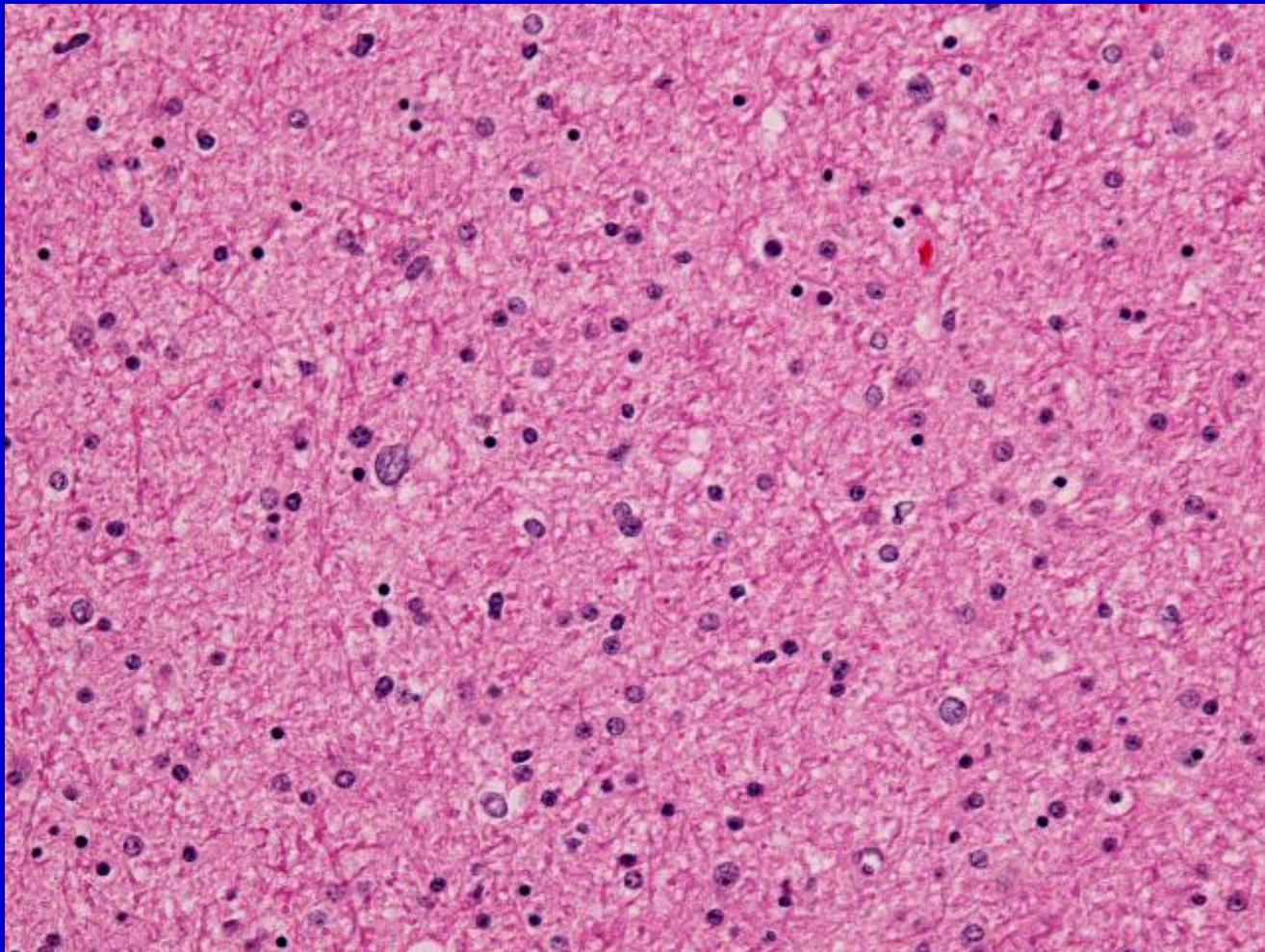
Tumor	Gender M/F	Age median (range)	IDH1R132H positive/total
Diffuse astrocytoma WHO II	33/20	36 (23–72)	44/53 (83%)
Anaplastic astrocytoma WHO III	11/20	38 (29–73)	25/31 (81%)
Oligoastrocytoma WHO II	4/6	37 (31–66)	9/10 (90%)
Anaplastic oligoastrocytoma WHO III	5/3	41 (32–77)	7/8
Oligodendrogioma WHO II	12/4	43 (33–65)	16/16 (100%)
Anaplastic oligodendrogioma WHO III	8/8	44 (30–78)	14/16 (88%)
Secondary glioblastoma	4/3	44 (33–58)	5/7
Primary glioblastoma	31/25	61 (33–94)	2/56 (4%)
Ependymoma WHO II	13/6	42 (1–66)	0/19
Ependymoma WHO III	23/24	9 (2–47)	0/47
Pilocytic astrocytoma WHO I	10/11	14 (2–56)	0/21
Meningioma WHO I	10/14	64 (31–88)	0/24
Medulloblastoma WHO IV	15/10	22 (1–61)	0/25
Schwannoma WHO I	7/5	55 (16–66)	0/12

Capper D, et al. *Brain Pathol.* 2010

脳腫瘍とIDH1/2

- クエン酸回路の酵素、アイソザイム
- ある種のグリオーマで点突然変異
- Astrocytoma, oligodendrogloma, oligoastrocytoma, secondary glioblastoma
- 変異部位は、IDH1の R132H (84%)
- R132H変異のIDH1に対する抗体開発
- mIDH1の免疫組織化学的検索が可能

Glioma vs Gliosis

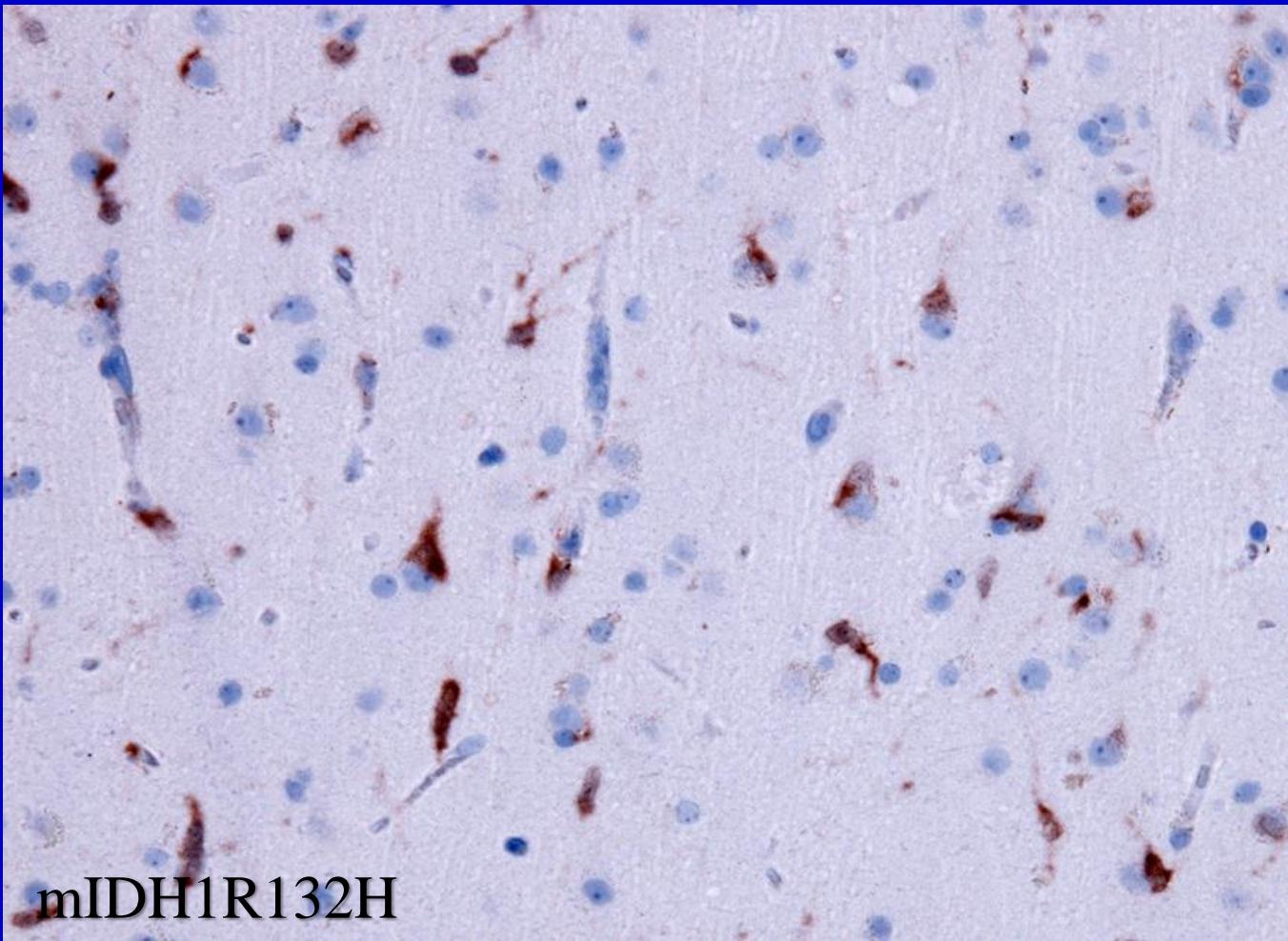


Glioma vs Gliosis

Underlying Lesion	n	mIDH1(+)	WT1(+)	P53(+)
Adjacent metastasis	29	0	2	25
Brain invasive meningioma	8	0	0	3
Vascular malformation	22	0	2	8
Ischemia	13	0	3	7
Hemorrhage	7	0	1	4
CNS malformation	2	0	0	1
Hippocampal sclerosis	9	0	2	4
Unspecific inflammatory infiltrates	10	0	3	9
Abscess	3	0	2	3
PML	6	0	3	6
Multiple sclerosis	8	0	1	4
Vasculitis	3	0	1	2
Total	120	0 (0%)	20 (17%)	76 (63%)

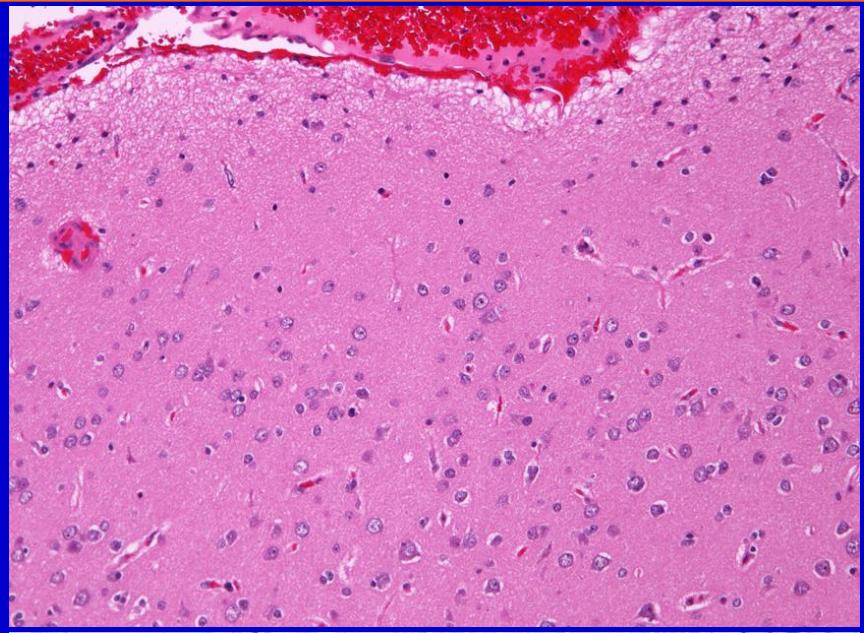
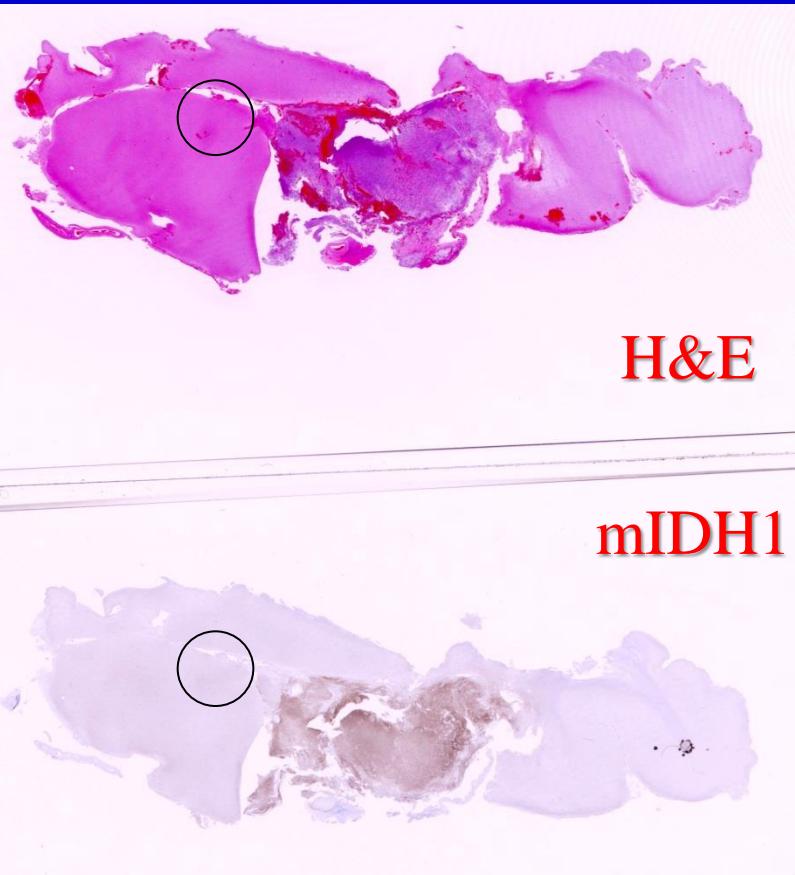
Capper D, et al. *Am J Surg Pathol.* 2010

Glioma vs Gliosis

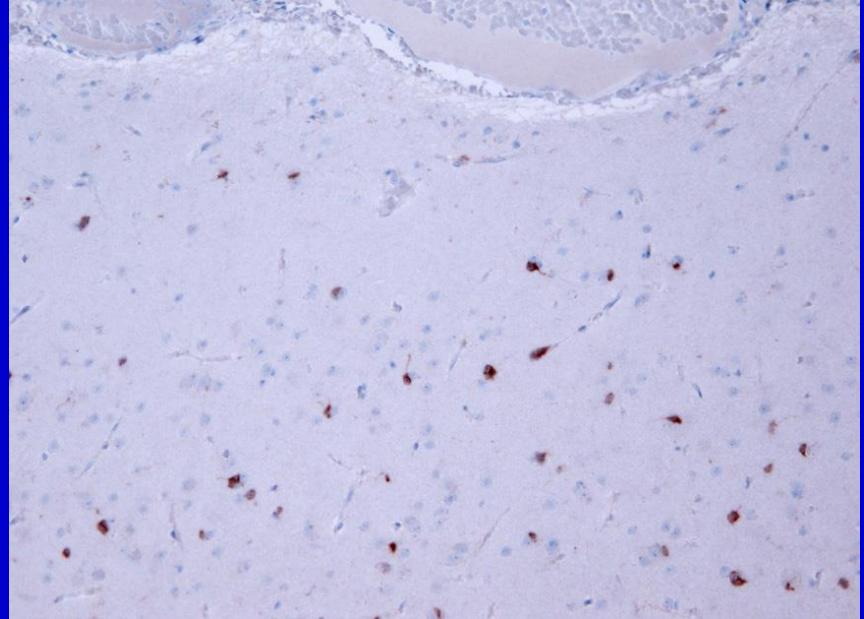


mIDH1R132H

Tumor marginの評価

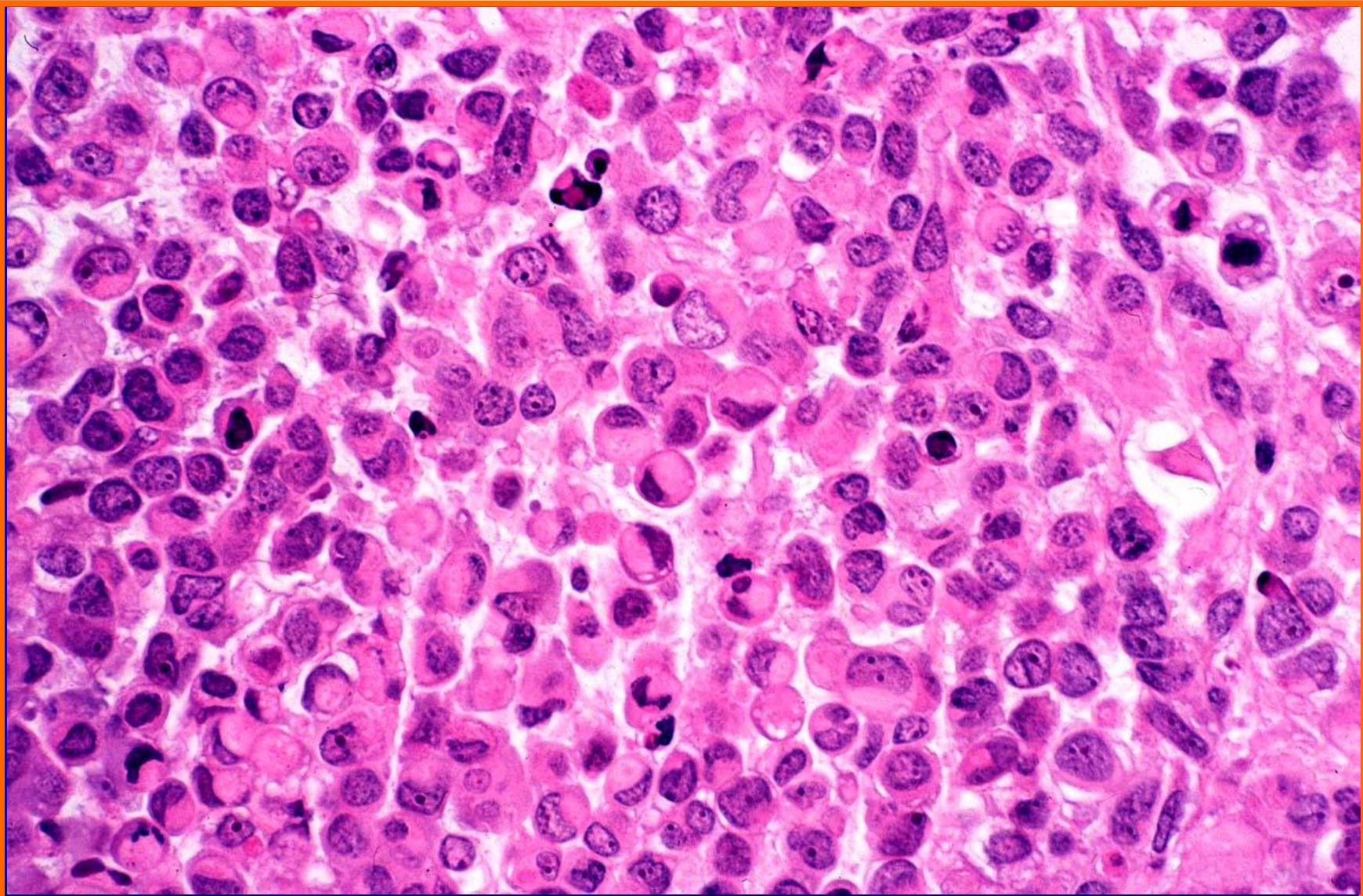


mIDH1



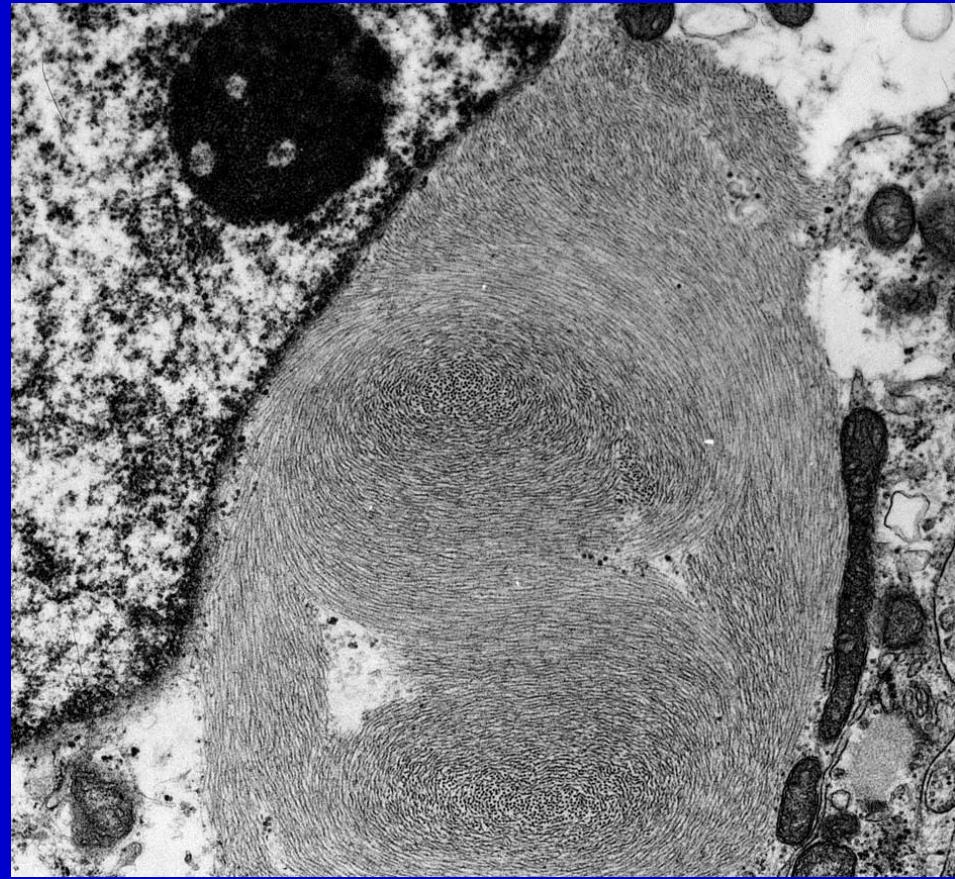
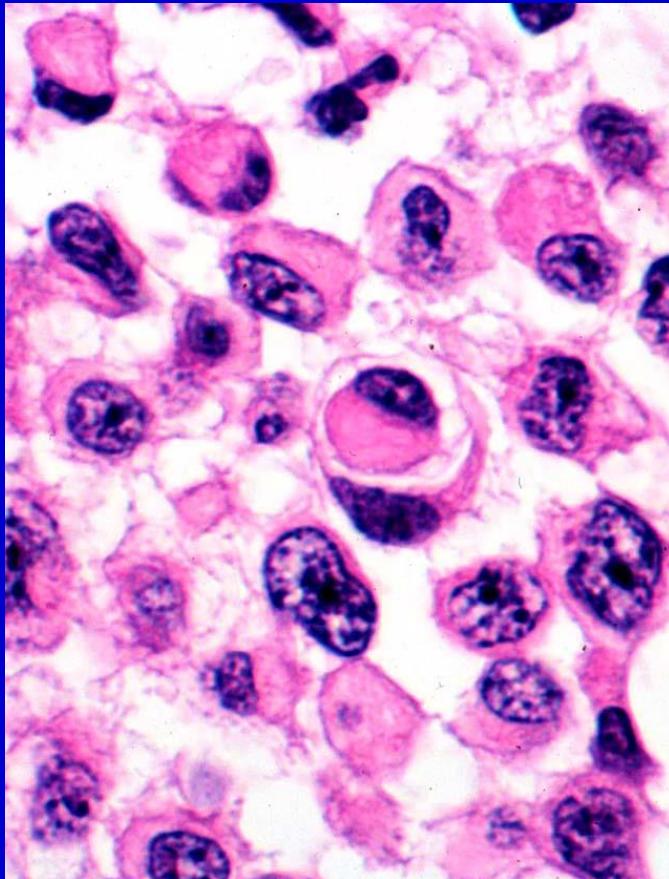
INI1

- *Integrase interactor 1 gene*
- Syn. *hSNF5, SMARCB1*
- Locus: 22q11.2
- Biegel JA (1999) : AT/RTでは*INI1*に変異が見られる。Cancer Res 59: 74-79
- Judkins AR (2004) : INI-1蛋白の抗体を用いてAT/RTの診断が可能。Am J Surg Pathol. 28: 644-650

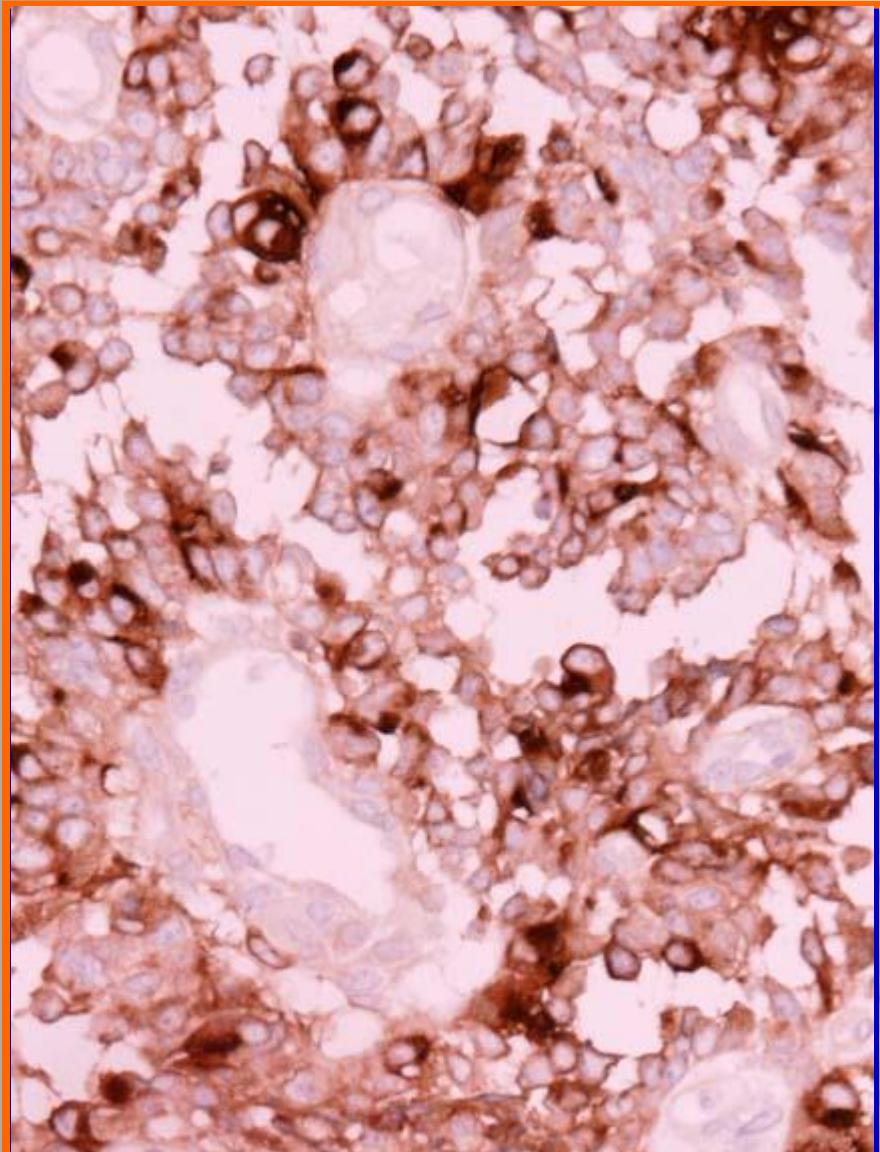


Atypical teratoid/rhabdoid tumor, H.E.

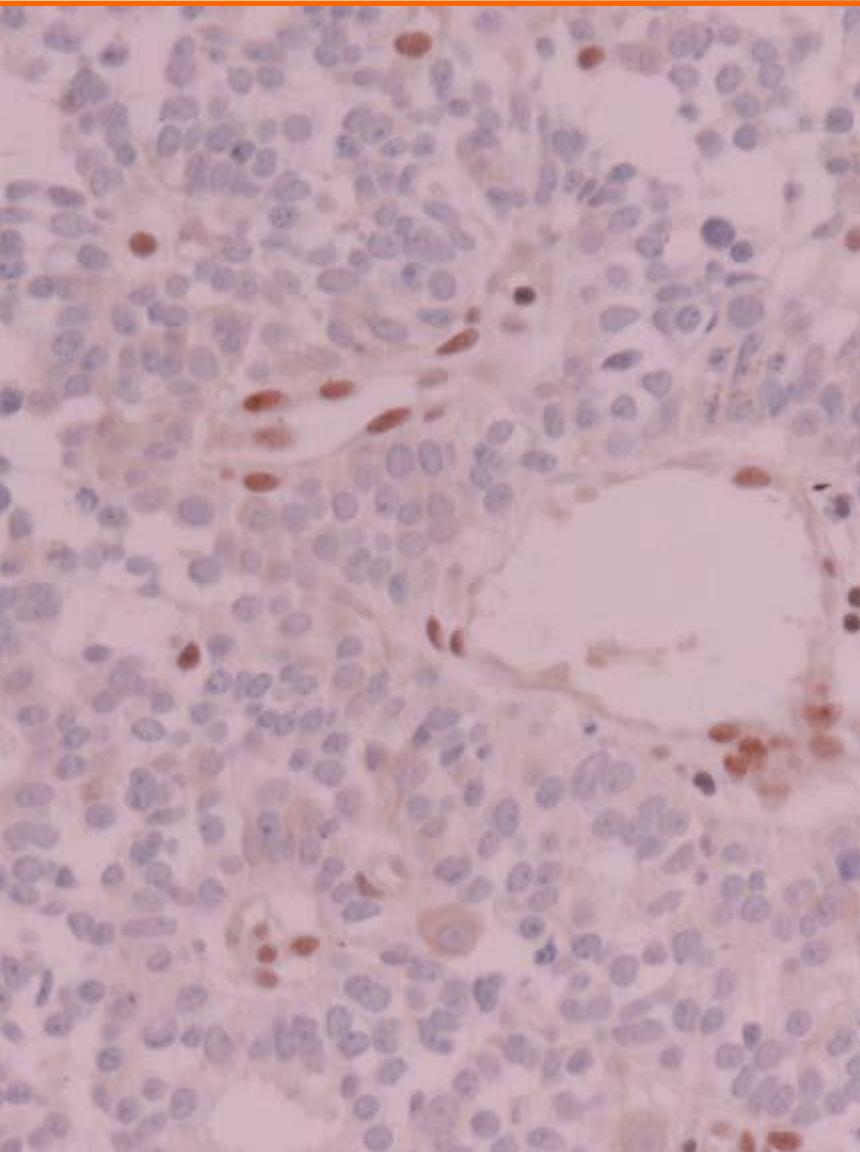
Atypical Teratoid/Rhabdoid Tumor



Rhabdoid cells with intracytoplasmic filamentous inclusion



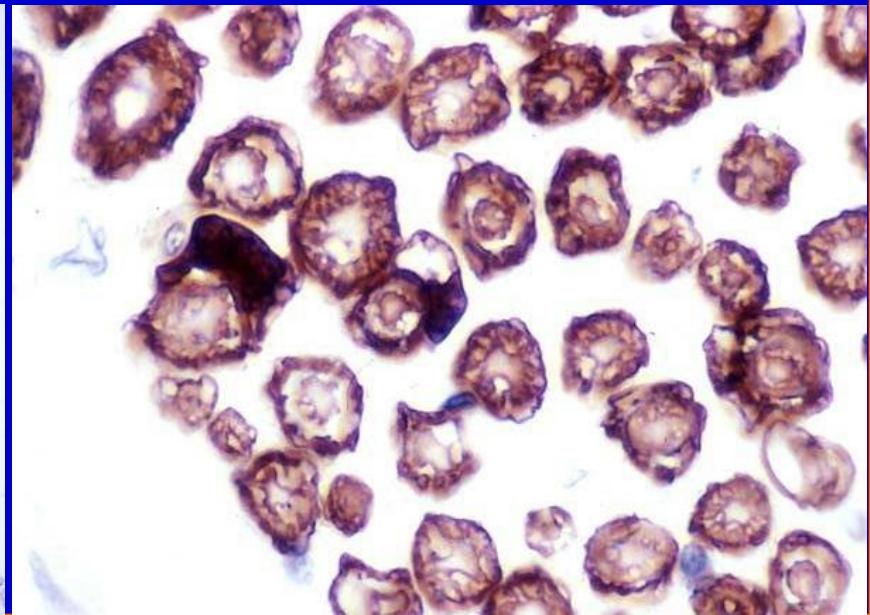
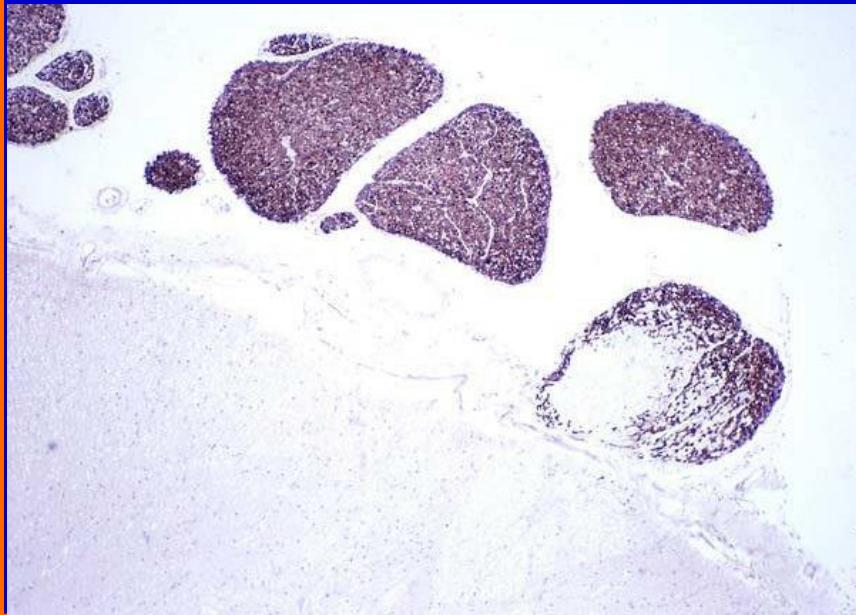
EMA

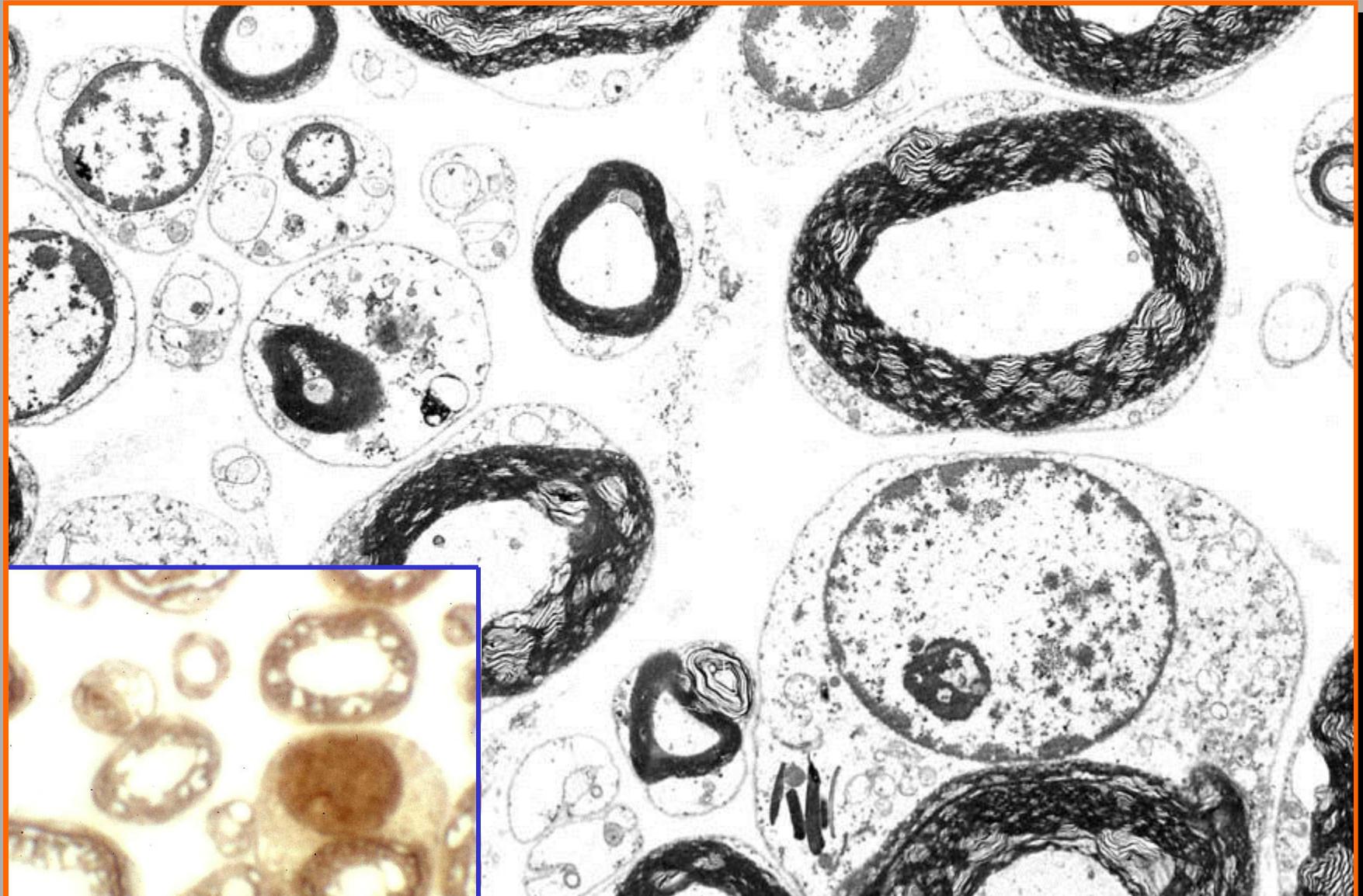


INI-1

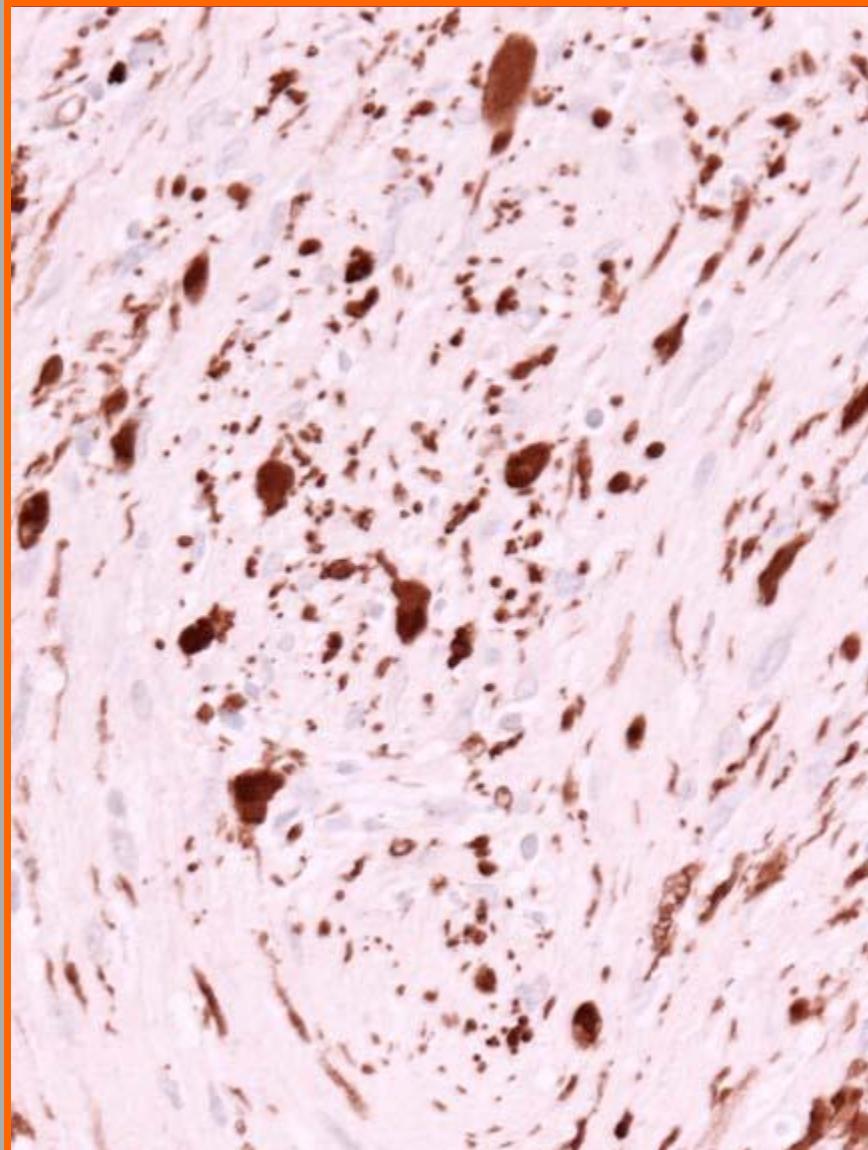
Schwann/2E

- 有髓性シュワン細胞と末性髓鞘を特異的に認識するモノクローナル抗体
- (コスモ・バイオ株式会社, Cat No.GU01M02ASA)

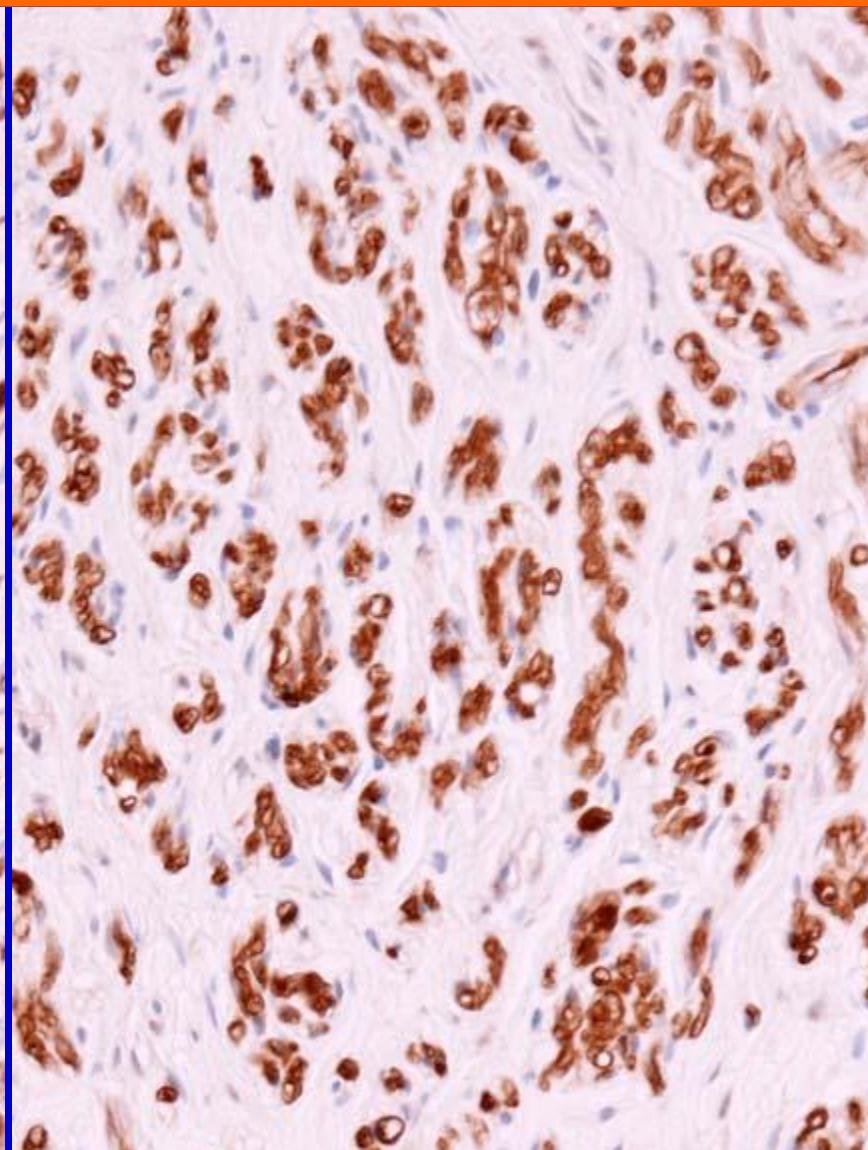




Schwann/2E ← Adjacent sections → EM



Schwannoma



Traumatic neuroma

Schwann/2E

- 末梢神経腫瘍における局在 *

Schwannoma	72/92 (78.3%)
------------	---------------

Neurofibroma	0/12 (0)
--------------	----------

MPNST	0/6 (0)
-------	---------

Traumatic neuroma	3/3 (100)
-------------------	-----------

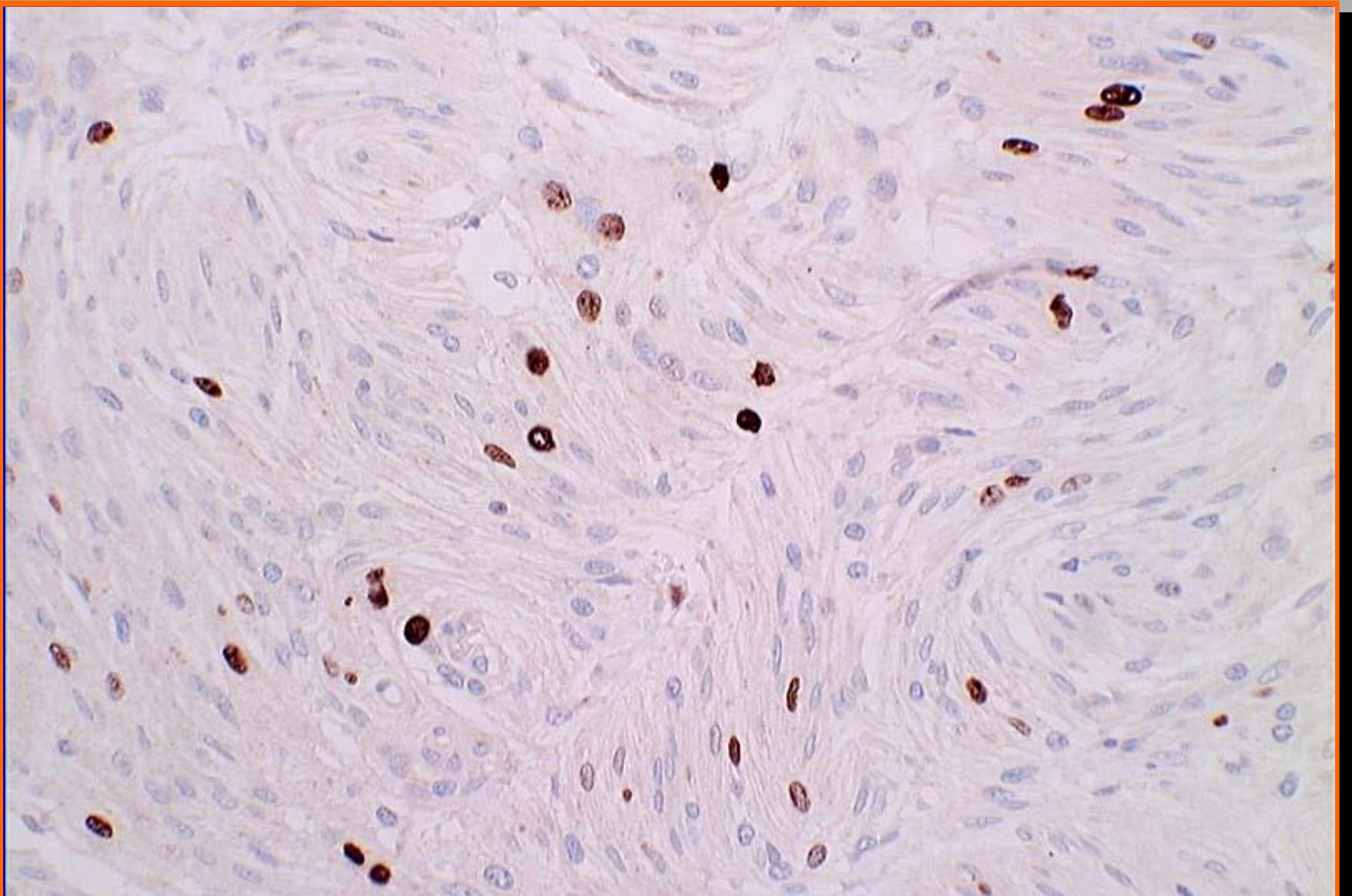
- その他の脳腫瘍 **

Glioma, meningioma, etc	0/339 (0)
-------------------------	-----------

*Arai H, 1998, ** Ikota H. 2006

細胞増殖マーカー

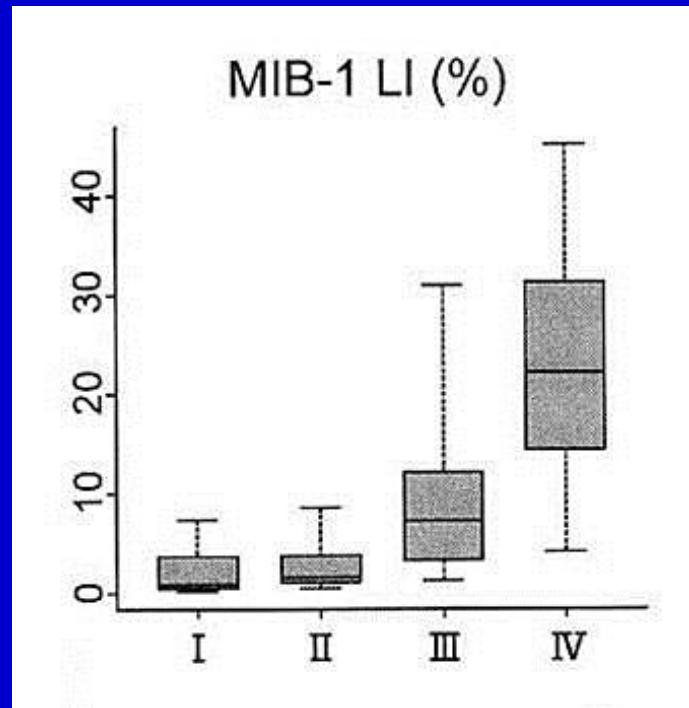
- Ki-67 (MIB-1)
 - Antigen: intranuclear proteins, 345 & 395 kDa
 - Positive for cells in late G1, S, G2 and M phase of the cell cycle
- DNA topoisomerase II α
- Proliferating cell nuclear antigen
- Thymidine-H³, BrdU



Atypical meningioma: MIB-1 staining

Ki-67 (MIB-1) index

- 脳腫瘍のgradingに
利用する試み
 - Astrocytoma
 - Oligodendrogloma
 - Ependymoma
 - Meningioma



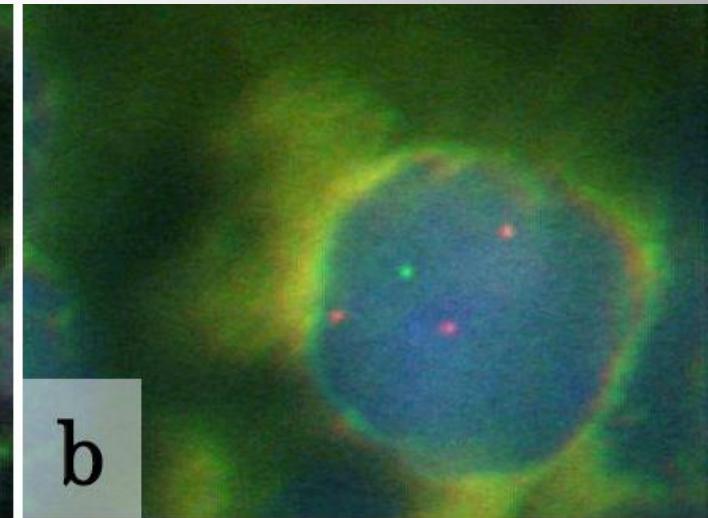
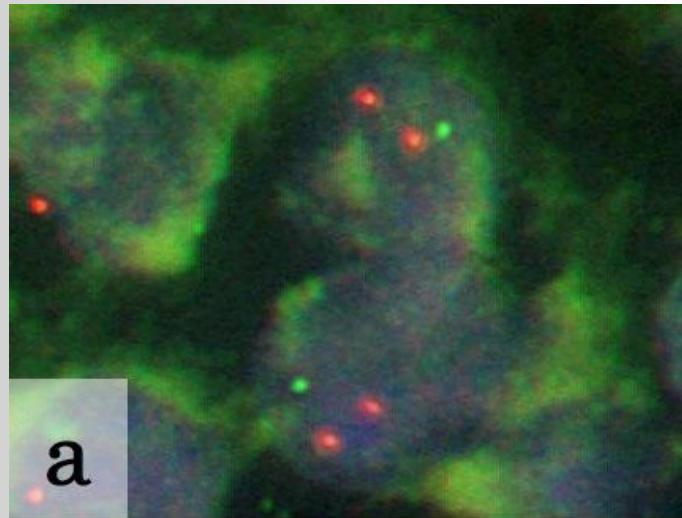
MIB-1 LI in astrocytomas
Tanaka G. 2004

FISH/CISH による解析

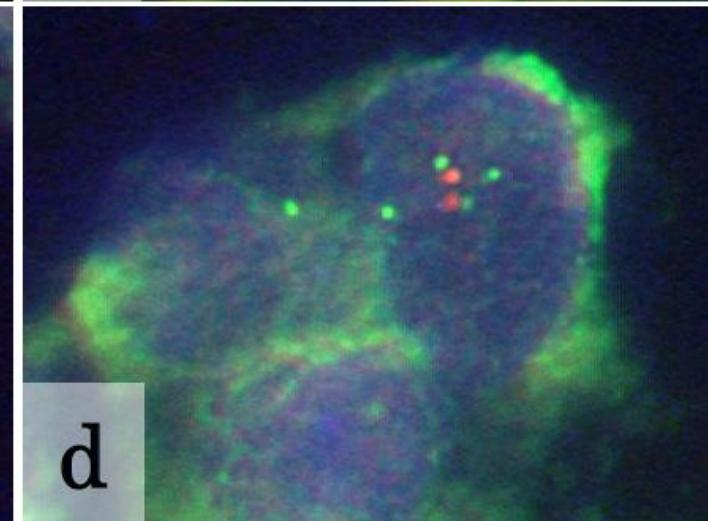
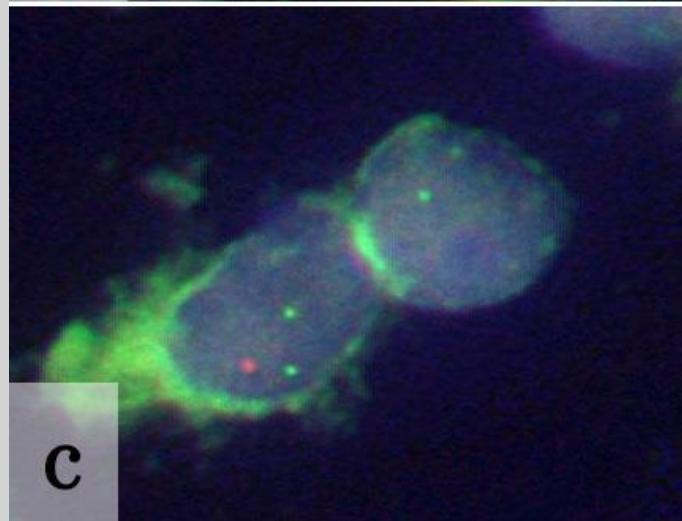
- 1p/19q codeletion oligodendroglioma
- *EGFR* amplification glioblastoma
- *PTEN* deletion glioblastoma
- *CDKN2A* deletion glioma
- *BRAF* rearrangement pilocytic astro.
- 22q11.2 deletion AT/RT
- 19q13.42 amplification ETANTR

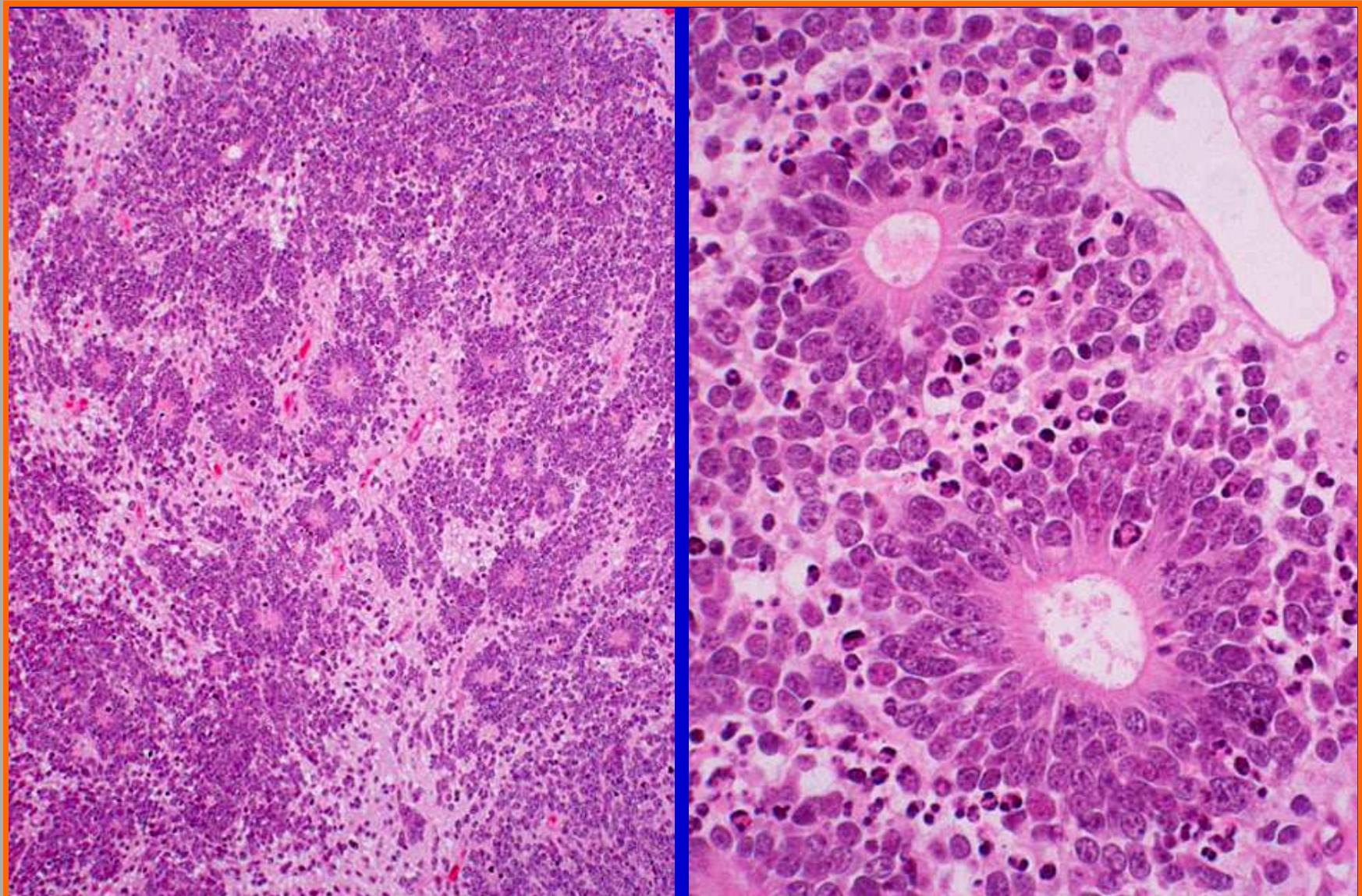
FISH analysis: oligodendrogloma cell and ganglion cell

1p36(green)
1q25(orange)



19p13 (green)
19q13 (orange)

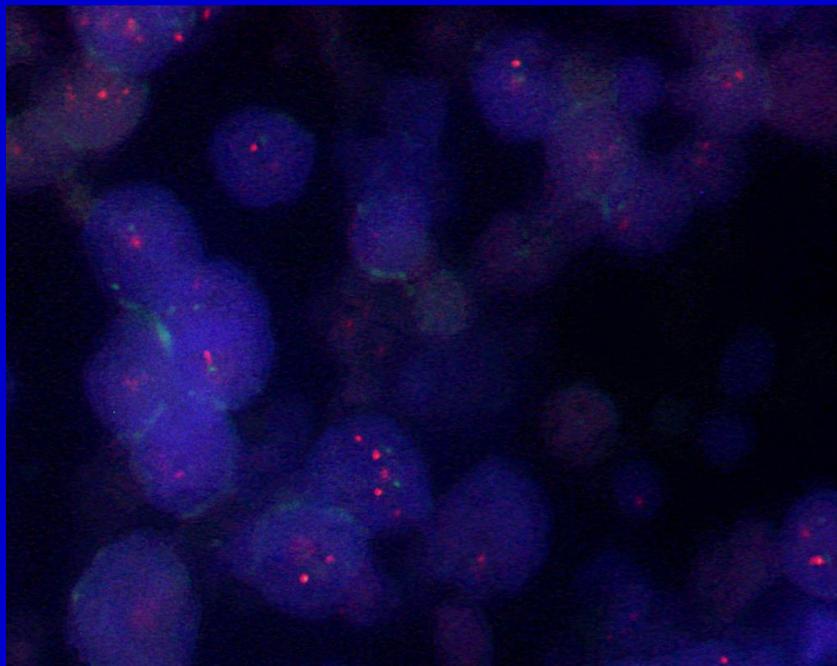




Embryonal tumor with abundant neuropil and true rosettes

FISH

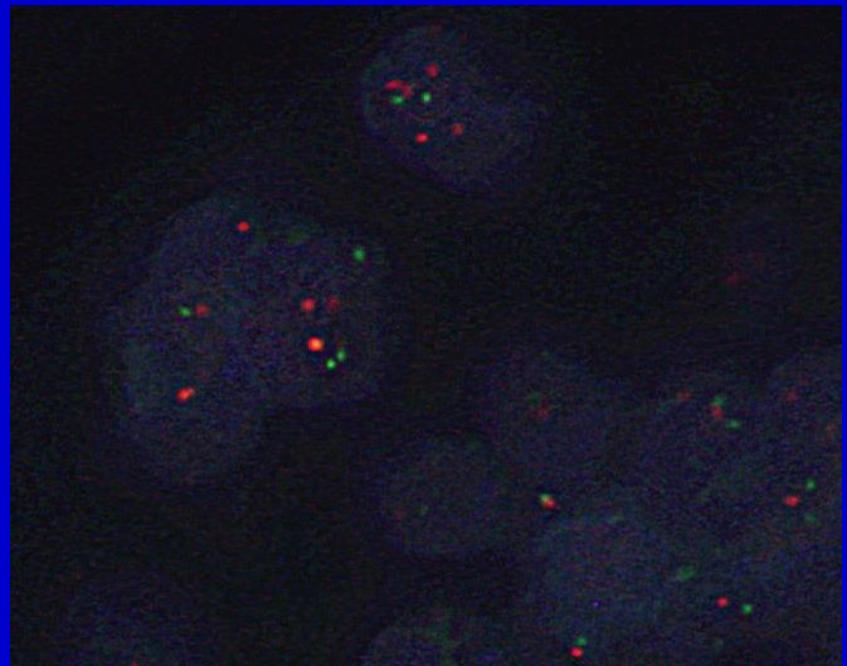
Case 1, ETANTR



Red: RP11-634C1
(19q13.42)

Green: RP11-451E20
(19p13.11)

Case 2, ETANTR



Red: RP11-381E3
(19q13.42)

Green: RP11-451E20
(19p13.11)

Nobusawa S, 2012

脳腫瘍の病理診断：要点

- 臨床情報、画像、手術所見で絞り込む
- 光顕像と免疫染色が決め手
 - 良い標本を（固定, 包埋, 薄切, 染色）
 - 標本の全体像から組織像, 細胞像へ
 - 間質の所見も役立つ：血管増殖像, 基質
 - 免疫染色は抗体の選択と使用法が重要
 - 増殖能の評価：Ki-67 (MIB-1), PHH3
- 細胞遺伝情報は有用、電顕は補助的

共同研究者

- 平戸 純子
- 横尾 英明
- 伊古田勇人
- 田中 優子
- 信澤 純人
- 松村 望
- 新井 秀雄
- 新井 基展
- 永石 雅也
- 鈴木 文
- 狩野(新井)華子
- 山崎 博子
- 横田 眞知子
- 磯田 浩二

第26回日本臨床細胞学会関東連合会学術集会
COI開示

筆頭演者名： 中里 洋一

今回の演題に関して開示すべきCOIはありません。